PREPARATION, CHARACTERIZATION, AND *IN VITRO* RELEASE OF ORAL DRUG DELIVERY SYSTEMS MADE FROM ZEIN AND PECTIN

TANG WAI WA

M.Phil

The Hong Kong Polytechnic University

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The Hong Kong Polytechnic University

Department of Applied Biology and Chemical Technology

Preparation, Characterization, and *In Vitro* Release of Oral Drug Delivery Systems Made from Zein and Pectin

Tang Wai Wa

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Certificate of Originality

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Tang Wai Wa

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Abstract

Controlled drug delivery has been an extensively studied field in the past decades. The advanced drug delivery systems bring higher compliance and convenience to patients. And more importantly, they improve drug efficacy and reduce side effects. Modified-release or delayed-release drug delivery systems are two major designs of the controlled release dosage forms. The modified-release dosage forms provide slow and continuous drug delivery, while the delayed-release dosage forms control the drug delivery to specific sites. These dosage forms can be made by different strategies. This work presents the use of plant-derived materials taking advantages of their natural properties to make a controlled release capsule or dosage form. Corn zein is a hydrophobic protein having unique solubility and other properties which are favorable for developing a delayed-release capsule. Pure zein capsule can be very brittle and this hinders its use in pharmaceutical industry. In this project, the mechanical strength of the zein capsule was improved by plasticization. The effects of three types of plasticizers, i.e. oleic acid, polyethylene glycol (PEG) and glycerol, on the mechanical behavior, water vapor permeability (WVP), and structural morphology of the zein films were investigated. The supramolecular structures of the plasticized films were characterized using scanning electron microscopy (SEM). Changes in the secondary structure of the films were analyzed using Fourier transform infrared spectroscopy

equipped with attenuated total reflectance (FTIR-ATR). Chemical interactions between the plasticizers and zein molecules were examined using differential scanning calorimetry (DSC). Water solubility and disintegration tests were also carried out for the zein capsules made. After characterization by the above tests, oleic acid-plasticized zein capsules were selected with satisfactory mechanical strength and good resistance to disintegration in liquid media. Dissolution studies of the capsules were performed showing that zein capsules have certain resistance to gastric digestion. Another plant-based material was also used in this project, which was pectin. Pectin is well-known for its indigestibility in the stomach and small intestine, and being degradable in the colon. However, its swelling property causes premature release making controlled delivery infeasible. Zein and pectin have very different behavior in their physical properties and digestibility. Combining zein and pectin has a potential in forming colon-specific delivery dosage forms. We proposed that zein and pectin formed complex by hydrogen bonding as observed from FTIR-ATR. Characteristic structures of the zein-pectin dosage forms were presented in the SEM images. From the swelling test, the swelling behavior of pectin was suppressed by zein in the zein-pectin interacted complex. Protection of zein against protease digestion by pectin was shown to be effective from the *in vitro* performance of drug release. These indicate that combination of zein and pectin may be a promising controlled drug delivery system.

Research Publications

Journal Papers

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Abbreviations

α	alpha
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflectance
β	beta
c	centi (1×10^{-2})
Da	Dalton
℃	Degree Celsius
δ	delta
DSC	Differential Scanning Calorimetry
FTIR	Fourier transform infrared spectroscopy
γ	gamma
GI	Gastrointestinal
L_0	gauge length
b_1	gauge width
T_g	Glass Transition Temperature
L_1	grip separation
HPLC	High Performance Liquid Chromatography

h	hour(s)
k	kilo (1×10^3)
M	Mega (1×10^6)
M	Molarity
μ	micro (1×10^{-6})
m	mini (1×10^{-3})
min	minute(s)
n	nano (1×10^{-9})
L_2	overall length
b_2	overall width
Pa	Pascal
PBS	Phosphate Buffer Solution
PEG	Polyethylene glycol
%E	Percentage Elongation
RH	Relative Humidity
s	second(s)
SEM	Scanning Electron Microscopy
SCF	Simulated Colonic Fluid
SGF	Simulated Gastric Fluid

SIF	Simulated Intestinal Fluid
SI	Swelling Index
TS	Tensile Strength
WVP	Water Vapor Permeability
w/w	weight-to-weight
w/v	weight-to-volume

Chapter 1 Introduction

1.1 Gastrointestinal physiology

Oral delivery is the most popular route of drug administration, which has higher patient compliance and greater convenience than other routes, like nasal, pulmonary, rectal and so on.[1-4] During oral delivery, drug and dosage forms pass through the gastrointestinal (GI) tract, and are subjected to physiological changes in different segments. Design of functional dosage forms requires clear understanding of the intestinal environment. In general, the drug delivery systems in GI tract are affected by various factors, including the fluid composition, transit time, pH and bacteria. These factors are further influenced by the physiological conditions of individuals, such as food ingestion, gender, age and disease state.[5-7] They can be hugely varied among individuals which is a challenge to design the dissolution test for oral dosage forms. The following describes the current finding of human gastrointestinal physiology and the influence of these variations on the passage of the dosage forms in the GI tract.

Through oral route of medication, dosage forms pass through the esophagus and reach the stomach. The pH, fluid volume and fluid composition of stomach are significantly influenced by food ingestion. At fasted state, stomach produces gastric juice which has typical pH values ranging from 1.5 to 3.[8-10] Since water is usually

ingested with the drugs, fluid volume in stomach increases and the surface tension is lowered.[8] Such low buffer capacity and surface tension are mainly due to small amount of pepsin. Small amount of bile compounds may be found in the stomach because of duodenal reflux.[8] After food intake, the pH will increase to about 4.5- 7.[8-10] This is usually governed by the amount and the composition of the meal. Gastric acid secretion is stimulated to reduce the pH to the original level and overcome the buffer capacity.[8-10] After 2 to 3 hour of food ingestion, the secretion rate of gastric juice decreases as a result of the feedback mechanisms. In addition, gastric transit time is another extensively studied factor which is highly dependent on the fasted or fed state. The time of drug residence in stomach is less than 2 hours at fasted state.[11] However, at fed state, the size and composition of the food have a huge impact of gastric-emptying rate. Therefore, such a large intra- and inter-subject variability makes gastric residence time hardly predictable. Fed stomach allows only liquids and finely dispersed particles to be emptied.[12-13] After complete digestion in the stomach, which may take several hours, the dosage forms that may be large and non-disintegrated will be transited to the small intestine.[7, 14]

Unlike the stomach, food ingestion has less significant impact on the physiological conditions in the small intestine. The small intestine can be further

classified into different segments, and they are duodenum, jejunum and ileum. They play different role in digestion, secretion and absorption. Thus, their compositions and pH values of the intestinal fluids change along the small intestine. Digestive enzymes and bile salt are secreted from the pancreas and the gall bladder respectively. They are mostly found in the duodenum and jejunum, while the ileum contains small amounts of these.[7] At fasted state, the small intestine fluid has average pH values ranging from 5.5 to 7.5 and the values rise along the small intestine. The pH values of duodenum, jejunum and ileum are 5.5-6.5, 6-7 and 7-7.5 respectively.[8, 10, 15-16] At fed state, a slight increase in pH of the duodenum is observed from 5.5 to 6, while the pH values of the distal regions are independent of the food intake.[7] Fluid volume should also be considered because studies show that at fasted state, 'fluid pockets' appears in the 'dry areas' in the small intestine, and the overall fluid volume is small.[17] It is possible that the dosage form locates in the dry areas and does not in contact with fluid.[17] Since the transit time of small intestine depends mainly on the motility controlled by the migrating myoelectric complex, it is relatively consistent and kept at 3-4 hours for individuals.[18] Nevertheless, the postprandial state causes accelerated intestinal flow rates in jejunum and ileum.[6, 19] This effect on the transit time of the dosage forms is not well understood. Moreover, the discontinuous behavior of the small intestinal transit in one individual makes it more complicated in implications.[20-21]

After passing through the small intestine, the dosage forms enters the colon. The colon contains large amount of bacteria, which produce enzymes that are able to degrade carbohydrates and sometimes proteins and peptides.[7] These materials will be broken down into short chain fatty acids,[22] lowering the pH of the proximal colon to about 5.5 to 7.8.[23-25] The resulting fatty acids are then neutralized and the pH values rise to around 6 to 7.3 along to the distal colon, depending on the physiochemical conditions of the individuals.[23-24] The intra- and inter-subject colonic pH variations can be large. In contrast to the stomach and the small intestine, colon contains extensively mixed meal. This is also one of the factors that makes it hard to predict the transit times of nutrients and drugs.[7] Similar to that in the small intestine, the transit in colon is not continuous, and the transit time in the colon varies largely from 6 to 48 hours, or even longer.[26] Therefore, the transit time of the dosage forms in the colon is unpredictable.

Delivery of dosage forms depends mostly on the fluid volume, composition, pH and transit time. In disease states, these physiological factors may alter. Fluid is more viscous due to larger water reabsorption in constipation patients. Also, there can be malabsorption of fat or bile salt in distal ileum of Crohn's patients.[27-28] These patients usually have accelerated intestinal transit time and uneven distribution of material in

the colon.[29] These result in reduction of the exposure time of drug at the disease site. It is found that the pH values of the small intestine are not affected by the disease state, but that of the colon are affected. A significant reduction of the pH of the colon is observed in the Crohn's patients. The pH value can drop to an average of 5.3 and as low as 2.3, compared to the healthy individuals having pH 6.8 to 7.2 in the proximal and distal colon respectively.[30] In addition, bacterial composition in the colon changes in diseased patients. Treatment using antibiotics or probiotics can modify the bacterial content, as well as the pH in the large intestine due to the production of short chain fatty acids.[6] This affects the drug release of not only microbial triggered dosage forms, but also the pH-responsive delivery systems. Such problems affect the performance of the dosage forms, causing difficulty in the development of drug delivery systems.

Understanding the gastrointestinal physiology can help develop an *in vitro* model to mimic the *in vivo* conditions. The pH value of the solutions is adjusted and enzymes and salts are added to the system to simulate the corresponding conditions in different segments of the GI tract when the oral dosage form passes through. The commonly adopted *in vitro* testing involves dissolution in 900-100 ml of buffer solutions (USP I-II). However, the actual fluid volume is smaller.[31] The mechanical force mimicking peristalsis of the intestine is also hard to be controlled by the system. And other factors,

such as the fluid dynamics, motility and transit, are influential.[6] A more realistic model, the TNO intestinal model (TIM) has been developed by controlling the various factors close to the *in vivo* conditions.[32] But it is still hard to predict and mimic the conditions of GI tract accurately because of limited understanding and poor characterization of the gastrointestinal environment, especially in the colon. It is challenging to develop an oral drug delivery that can overcome the intra- and inter-subject variabilities.[26, 33]

1.2 Controlled drug delivery

In order to control the drug concentration and stability, carriers are often used to protect the drug from the changing environment.[34] Such carriers are usually made in the form of capsule, hydrogel, microsphere or nanoparticle. They provide not only protection against oxidation and hydrolysis, but also functional properties, such as controlled release. Controlled drug delivery is an advanced technology which determines the way of drug release based on two principles. One is to control the drug release rate from the carrier and maintain the drug concentration at the therapeutic level over a period of time; the other is to trigger the drug release at the targeted site so that the systemic drug concentration is low, while the concentration at the site is high.[35] These offer potential advantages of enhanced drug efficacy, effective control of drug levels, decreased dosing frequency, and reduced side effects.[35-37]

The development of gene therapy,[38] and the production of protein and peptide drugs, lead to considerable attention on drug delivery system. Therefore, controlled drug delivery system plays an important role in pharmaceutical industry. Gastric passage is critical for protein drugs and gene delivery because they are vulnerable to protease attack and acid hydrolysis in stomach.[39-40] Controlled release to small intestine is needed to prevent gastric digestion and consequently improves their oral

availability and stability. In addition, drug delivery for treatment of colon related diseases, such as colorectal cancer and inflammatory bowel disease, is another potential application. Chemotherapy drugs and anti-inflammatory drugs are often required for disease treatment. However, these drugs cause serious side effects of a high dose. Long-term use of these drugs is usually avoided. With the development of colon-specific delivery, these problems may be resolved as the total amount of drug administrated can be reduced.

1.3 Colon-specific drug delivery systems

Colon-specific drug delivery systems can be categorized based on their controlled release approaches. In the GI, gastric and intestinal fluids have distinct pH, enzyme composition, and luminal pressure. Taking advantages of these differences, promising drug delivery systems have been designed to control degradation of the carriers. They are classified as time-based, pH-based, enzyme-based, and pressure-controlled delivery systems. To deliver the drug to the distal ileum and colon effectively, a combined use of these systems may be needed.

1.3.1 Time-based delivery systems

The rate of drug release of a time-based delivery system depends on permeability of carriers and their thickness.[41] In general, the capsules prevent and control the drug release for 3-4 hours after gastric emptying. The drug release location mainly depends on the transit time of the GI tract.[42] Transit time in small intestine is relatively constant at 3-5 hours while transit through colon can be up to 78 hours.[41, 43] However, there is significant variation of transit time in stomach between individuals, which ranges from 0.60 to 4.52 hours.[44] In order to ensure that the capsules pass through the stomach without drug release, enteric materials are coated on the capsules. The enteric coatings allow drug release in small intestine by a change in pH, osmotic pressure, or swelling

behavior of the materials, resulting in rupture of the coatings.[43]

1.3.2 pH-based delivery systems

Due to the intra- and inter-subject variation of gastric residence time, pH-based drug delivery systems are developed on the basis of the solubility or digestibility of carriers at different pH values. The drug release mechanism relies on the pH in the GI tract. Enteric coatings protect capsules against gastric digestion with pH around 1.2 in the stomach. When the drug reaches the small intestine, enteric polymers dissolve in pH 6-7 intestinal fluid, allowing drug release.[45-48] Targeted drug delivery to small intestine can be achieved. Nevertheless, pH-based delivery systems may not be a promising method for colon-specific delivery because of slight difference in pH between small intestine and colon, and inconsistent pH in colon.[42-43, 49] In order to develop a colonic drug delivery capsule, time-based and pH-based delivery systems are combined by increasing the thickness of the coatings.

1.3.3 Enzyme-based delivery systems

Enzyme-based delivery is another popular controlled release approach, which uses the enzymes produced by microflora in the targeted site to trigger the drug release. A large amount of microflora is mainly found in the colon. Various enzymes are

produced, such as azoreductase, glycosidases and esterases by the bacteria. Therefore, enzyme-based systems are often developed for colon-specific drug delivery.[37, 50-51] Prodrugs, hydrogel and biodegradable polymers are used to develop colon-specific capsules. Polymers are cross-linked with azo groups and glycosidic linkages, so that they can be degraded in colon by the corresponding enzymes.[51] For biodegradable polymers, polysaccharides are often used to form films and hydrogels. Non-starch polysaccharides are not digestible in stomach and small intestine, but they can be decomposed in colon by enzymes.[42] Enzyme-based delivery is considered as one of the most promising strategies for colon-specific delivery. However, polymer swelling, which leads to premature release, and possible toxicity of the degraded products are the major concerns of the formulation design in enzyme-based systems.[42-43]

1.3.4 Pressure-controlled delivery systems

Pressure-controlled release is an alternative for colon-specific drug delivery. Peristalsis in the distal colon induces high luminal pressure, leading to rupture of the drug carriers and then drug release.[43, 45, 49] Pressure-controlled capsules can be formed by coating a layer of ethylcellulose inside a gelatin capsule. As gelatin dissolves in the upper GI tract after oral administration, the ethylcellulose layer forms a balloon encapsulating drug solution. Reabsorption of water in the colon and increase in

viscosity of the luminal content result in a high luminal pressure. Such a high pressure causes the breaking of the balloon and consequently the drug is released.[52]

1.4 Zein

Corn is one of the major agricultural product, having high annual production in America. It is processed for the production of starch, oil and ethanol, resulting in a large amount of protein byproducts.[53] After wet milling, corn gluten meal remains and it is usually treated as animal feed. In addition, protein byproducts can be further extracted and refined with additional value. Corn is rich in a valuable protein called zein, which has unique properties and can be modified for various use. Zein is mainly distributed in the endosperm. It performs mainly as a nitrogen storing protein in the developing seed. It is deficiency in a number of essential amino acids, such as lysine and tryptophan which limits its use in human food.[53] Thus, zein is more commonly used for industrial purposes.

Zein is classified as a prolamine and more than 50% of the amino acid residues are nonpolar.[53] The amphiphilic nature of zein leads to its unique solubility. In general, zein is insoluble in water or ethanol, but soluble in 50-95% ethanol-water mixture.[53-56] It is a mixture of peptides having different molecular size and physical characteristics, and can be classified as α, β, δ and γ-zein.[53, 57] They can be fractionated based on their different solubility. It is found that α-zein accounts for approximately 35-70% of the total zein.[53] It is the major product present in commercial zein because

the other types of zein are lost after the reducing and recovery process during extraction. α-Zein can be further classified into two sizes which have molecular sizes of 19 kDa and 22 kDa. Studies also show that α-zein is beneficial and stable while the other types of zein cause gelation that is undesirable for applications.[53] As demonstrated by the measurements of circular dichroism and optical rotatory dispersion, zein has an α-helical content of 50-60%. A helical wheel model of α-zein in solution is suggested, indicating that the antiparallel packed α-helices are joined by glutamine-rich bridges.[55, 58] Apart from the α-helical structure, β-sheet is also formed as shown in infrared studies because conformational changes occur with changes of the solvent. As the solvent becomes more hydrophilic, α-helix is unfolded and transformed into β-sheet. This β-sheet induces the transition of the adjacent α-helices to form a β-sheet pleated structure.[59]

The most commonly used solvent of zein is aqueous alcohol. As ethanol evaporates faster than water from zein solution, the solvent becomes more hydrophilic and results in evaporation-induced self-assembly of zein. Zein molecules tend to attract each other through the hydrophobic interactions. Various structures can be obtained by zein self-assembly, such as spheres, sponges and films, depending on the zein concentration in the solution. At high zein concentration, microspheres fuse

together to form a film.[60] Zein films are solvent and grease resistant, and their hydrophobic character is preferable for industrial applications, such as flavor masking, water vapor barrier coatings, biodegradable films, and textiles.[53, 61] However, unmodified zein films are brittle and the hygroscopic nature of zein make them less favorable for packaging applications. Plasticizers and crosslinking agents are often added to improve the mechanical properties of the zein film. In general, plasticizers can be classified according to their plasticizing effects as primary or secondary types. Primary plasticizers, such as fatty acids, glycols and esters, can be used alone having improved mechanical properties. Whereas secondary plasticizers, like glycerol and sorbitol, have poor plasticizing effect when used alone. But they are effective when combined with the primary plasticizers.[57] Water is also considered as an effective plasticizer of the zein film. Because zein is a hydroscopic material, it will gain and lose moisture from the surrounding. Thus, the flexibility of the film is seriously affected by the relative humidity (RH).[57] In the past, formaldehyde was added as a crosslinking agent to enhance the tensile strength of the zein film.[62-63] However, due to the toxicity of formaldehyde, non-formaldehyde crosslinking agents, for example, polycarboxylic acids, are studied. Citric acid is found to be an effective crosslinking agent of the zein fiber.[64]

In pharmaceutical industry, zein can be used to form nanoparticles, microspheres, and hollow capsules with controlled release properties.[53-54, 65] It is known that zein exhibits certain resistance to pepsin digestion, resulting in low availability in the stomach.[66] But it can be digested by the proteases in the small intestine. And unlike many natural polymers, there is no premature drug release from zein carrier because it is not water-absorbing. Thus, zein can protect the drug through the stomach and allow its release in the intestine to achieve targeted delivery.

Zein is commercially extracted from corn gluten meal using polar solvents, such as isopropanol, with the addition of sodium hydroxide. Nonpolar solvents are used to remove color pigments and fats from the raw materials.[57] Corn itself is an abundant and inexpensive agricultural product. However, use of a large amount of solvents, high energy demand processes and low yield during extraction make zein a high-valued product which costs 10-14 USD per kg.[53] Cost effective extraction and purification methods of zein have yet to be found. The high cost of zein is also due to the low demand and therefore low production. The extracted zein appears as yellow in color due to the presence of xanthophylls, carotenoids and so on.[53] The color of zein hindered its applications. Removal of the color requires large amount of solvents and this further increases the cost of zein production. These problems result in limitation

of industrial utilization of zein and makes it not possible to compete with synthetic materials in the market.

1.5 Pectin

Pectin is a natural occurring water-soluble polysaccharide present in the cell wall of higher plants. Pectin is present mostly in the middle lamella in the cell wall. It accounts for 2-35% of the primary cell walls.[67] It is considered that pectin is retained in the cell wall by forming ionic bonds with calcium, and/or covalent bonds between pectin and hemicellulose. Pectin helps maintain the structure and firmness of soft plant tissues by acting as a cementing agent as well as a hydrating agent.[68] Apparently, pectin is a rich natural resource. The commercial manufacture of pectin is actually very limited. Pectin extracted from different sources may have different gelling properties for specific applications.[68] The raw materials should have sufficient quantity to ensure feasible pectin production. Nowadays, pectin is extracted from apple pomace and citrus peels. Pectin composition may be different depending on the environment factors and extraction conditions.[68]

Pectin is defined as a group of polysaccharide which is rich in covalently linked galacturonic acid (GalA).[67, 69] Generally, all pectin species contain *O*-1 and *O*-4 linked GalA. There are three main polysaccharide domains in all pectin, and they are homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II).[67, 69] HG is the most abundant pectic polysaccharide. It is a linear

homopolymer composed of α-1,4-linked D-galacturonic acid, containing 100-200 GalA residues and having 70-80% GalA methylesterified at the C-6 carboxyl. RG-I and RG-II are considered to be structurally more complex than HG because they have various side chains linked to GalA and rhamnosyl residues.[67, 69] The second most abundant polysaccharide in pectin is RG-I which accounts for 20-35% of pectin. It is glycosidically linked to the HG domains. It has the backbone of the disaccharide repeat α-D-GalA-1,2-α-L-Rha-1-4.[67] About 20-80% of the rhamnosyl residues in the backbone of RG-I are linked to side chains containing α-L-Ara*f* and β-D-Gal*p* residues. RG-II has the most complex structure in pectin. It has a HG backbone but with branches attaching to the GalA residues that are 1,4-linked. The side chains of RG-II contain 12 different sugars and 20 different linkages.[67] The conformation of the pectic polysaccharide domains can be analyzed using X-ray fibre diffraction, circular dichroism and NMR spectroscopy. The experimental results demonstrate the conformation flexibility of HG and association of HG with calcium ions. It is found that the rhamnose residues in the RG-I component do not have significant interruption on the HG chains, but exhibiting similar extended conformation as HG.[69] RG-II is a good candidate for analyzing the secondary structure of pectin. However, due to the complexity of the monosaccharide composition, complete modelling studies on the 3-dimensional structure are not well developed.

The water solubility of pectin depends on various factors, including the degree of polymerization and the number and distribution of methoxyl groups. Physical factors, such as solution pH, temperature and the concentration of solute, also have significant impact on the solubility. In the presence of calcium ions, sugar or acid, pectin is able to form gels as a result of the formation of a 3-dimensional network, which makes it important in applications. The gelation property of pectin depends on the degree of methoxylation.[68] Pectin can be classified into low methoxyl and high methoxyl types, and they exhibit different gelation mechanisms. The gelation of low methoxyl pectin is based on the ionic interaction between calcium ions and the carboxylic groups of the pectin at higher pH values. Dissociated carboxylic groups are cross-linked to form salt-like calcium bridges. An egg box model is proposed involving the formation of junction zones from the unbranched nonesterified galacturonan blocks.[68-69] Thus, the flexibility of the junction zones depends on the amount of reactive carboxylic groups and degree of methoxylation. A higher degree of methoxylation reduces gel strength. At lower calcium ion concentration, addition of sugar promotes gelation because of its hydrophobic effects and decrease in water activity of the pectin solution; at excess calcium level, sheet-like aggregates are formed with weakly bound calcium ions and the increased cross-linking destroys the gel leading to precipitation of pectin.[68] The gel formation of high methoxyl pectin is not based on the ionic interactions with

calcium ions. Instead, the junction zones are formed by cross linking between pectin molecules by hydrogen bonds and hydrophobic interactions. The conformation stability is controlled by the 3-dimensional hydrogen-bonded structure of water. The methoxyl groups form hydrophobic interaction with each other. The hydrophobic interaction is one of the major driving forces that determine the gelation ability and the strength. The presence of solutes, such as sugar, can change the magnitude of the interaction.[68] In addition, temperature, pH and pectin concentration and the degree of methoxylation are other factors that impose marked effect on the firmness of the gel.

Pectin has been used in human food as additives. It functions as a gelling agent, thickener, stabilizer, and emulsifier. The major food types that use a large amount of pectin are jams and jellies. Commercial production of jams and jellies adds dry pectin powder in the process, and sugar may be added to promote gelation of pectin. Pectin is also used in the manufacture of confectionery products, frozen barriers, beverages and so on.[68] It provides barrier properties, stability and texture to the food products. Moreover, the application of pectin in pharmaceutical industry is extensively investigated. Studies have demonstrated that pectin can lower the cholesterol levels in blood. There is also evidence showing that pectin causes less absorption of food by preventing the contact of the intestinal enzyme with the food.[68] Furthermore,

researchers have explored the use of pectin in developing controlled drug delivery. From the studies on human subjects and dogs, pectin is not digestible in stomach and small intestine and will be degraded in colon by bacterial enzymes.[34] Taking advantage of this property, pectin is used to form a drug delivery system.[70-71] However, due to its water solubility, it swells in the GI tract, leading to premature release of drug.[34] In order to reduce the water solubility of pectin, calcium pectinate can be added to form a better shield.[72] The addition of calcium pectinate is limited to low water soluble drugs. When pectin is mixed with chitosan, premature drug release is reduced in comparison to using pectin alone.[1]

The currently used gelatin capsule is derived from animal bones, tendons, and leathers. It is digestible in the upper GI tract and collapses during digestion. The commercially available gelatin capsules are coated with enteric polymers, such as Eudragit® L, S, and ethylcellulose to achieve controlled release.[41, 49] However, the safety and ethical concerns of gelatin capsule are raised due to the incident of chromium-tainted capsule made from unwanted leather.[73] Researchers are exploring new materials, such as hydroxypropylmethyl cellulose, starch, and guar gum, to make capsules.[45, 74] But these capsules cannot fulfill the necessary criteria of a capsule due to the high cost and unknown toxicity of semi-synthetic materials as well as the

swelling properties of the polysaccharides. The focus of research on zein or pectin is mainly on the formation of films, hydrogel, microspheres and nanoparticles. Research shows good flexibility of the films and controlled release properties of the delivery systems. However, the formation of hard capsules or solid dosage forms using these materials is rarely discussed. Only a few researchers have evaluated the combined effect of zein and pectin. Liu and coworkers[75] made pectin/zein hydrogel beads showing considerable improvement of the controlled release property. They proposed that zein suppressed the swelling behavior of pectin and pectin protected zein from protease digestion. Thus, colon-specific drug delivery could be achieved. Combining zein and pectin seems a promising drug delivery system.

1.6 Objectives

In this project, zein and pectin are selected to form the capsule for targeted drug delivery. They are biodegradable, biocompatible, and non-toxic natural polymers. But they are different in solubility, digestibility in GI tract and hydrophobicity. In view of these differences, controlled release at targeted sites may be achieved by a combined use of the polymers. Other studies on combined use of zein and pectin mainly focused on hydrogel beads and nanoparticles. Targeted drug delivery was achieved by combining zein and pectin to form a complex and it protected pectin from water

absorption. Zein coatings prevented premature release of pectin by suppressing the swelling behavior. Hard capsules formed from these natural polymers are not thoroughly investigated. The objective of this work is to develop plant-based targeted drug delivery systems using zein and pectin. Zein itself has the controlled release property which is desirable for the development of site targeting drug delivery systems. Hard capsules made from zein were plasticized by different compounds. Their mechanical properties and disintegration performance were investigated. The most favorable formulation was selected. Zein-pectin coated dosage form was also developed for colonic drug delivery. The interactions between zein and pectin were studied. The structure of the dosage form was characterized and dissolution studies was performed. Vitamin C was selected as a model drug because it acted as an antioxidant to prevent and inhibit colorectal cancer by neutralizing carcinogenic substances. It can also reduce the adverse effect of chemotherapeutic agents and enhance the efficacy of certain cancer drugs.[76-77] Vitamin C was sensitive to air, light and moisture, film coating was essential to protect it against decomposition.

In Chapter 2, the details of the experimental work of this project are presented. Chapter 3 describes the effect of different plasticizers on the zein films and characterization of the zein hard capsules. The *in vitro* performance and structural

morphology of zein-pectin dosage form are shown in Chapter 4.

Chapter 2 Materials and Methods

2.1 Chemicals

Zein was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (96% v/v) was from Guangdong Guanghua Sci-Tech Co., Ltd (Guangzhou, China). Oleic acid (OA, technical grade, 90%) was obtained from Aldrich Chemical Co. (St. Louis, MO, USA). Polyethylene glycol compound was obtained from Sigma Aldrich (Molecular weight 15,000-20,000, St. Louis, MO, USA). Pectin was purchased from Chengdu XiYa Chemical Technology Co., Ltd (Chengdu, China). Glycerol was from VWR International S.A.S (Radnor, PA, USA). Vitamin C tablets (Chewable Vitamin C, Tangy Orange, 500 mg) were obtained from Jamieson Laboratories Ltd (Toronto, Canada). All other chemicals were of analytical grades.

2.2 Instruments

Vitamin C concentrations were determined using HPLC-DAD system (Agilent Technologies 1200 Series, Santa Clara, CA, USA) with a RP-18 column (LiChrosorb® 250 mm × 4.6 mm, 5 μm particle size, W. R. Grace & Co.-Conn., USA). FTIR analyses were performed by Nicolet iS50 FT-IR Spectrometer equipped with iTR ATR unit (Thermo Fisher Scientific Inc., USA). Glass transition temperature (T_g) was measured by TGA/DSC (Netzsch STA 449C, Jupiter). SEM images were obtained using JEOL JSM-6490 SEM (Tokyo, Japan).

2.3 Preparation of zein films

A zein solution was prepared by dissolving 7 g of zein powder in 20 ml of 80% ethanol solution. Oleic acid, PEG and glycerol were added respectively in the zein solution at the weight percent of 7, 10, 15, 20, 30, 50 and 70 to zein. The solution was heat at 60 ℃ for 30 min with gentle stirring and then cooled to room temperature. Zein films were made by pouring 18 g of the solution onto a plastic dish with a dimension of 150 × 220 mm. A control sample was prepared under the same condition, except the addition of plasticizers. Gelatin films were prepared in the same way as the zein film with the addition of 15 (w/w) % of glycerol to gelatin. The films were conditioned at 50% RH for at least 48 hours before measurement.

2.4 Preparation of zein capsules

A zein solution was prepared as described previously in Section 2.3. Briefly, 70% (w/w) of oleic acid was added to the zein solution and heated at 60 ℃ for 30 min with gentle stirring. Stainless steel pin bars of size #0 cap and body (Safrroys Machines, India) were used in capsule making. The pin bars were brushed with a release agent which was composed of paraffin oil, oleic acid and lecithin, prior to dipping of the zein solution. The pin bars were then dipped into the zein solution for 5 s. They were taken out and spun for 30 s to form a uniform coating. The coating on the pin bars was

dried at 25 ℃. After drying, the capsules were removed from the mold, and trimmed manually to regular lengths. They were stored in a desiccator with silica gel for further measurements.

2.5 Preparation of zein-pectin coated dosage forms

Another zein solution was prepared by dissolving 2 g of zein in 10 ml of 80% ethanol and 1.4 g of oleic acid was added to the solution. The solution were heated with gentle stirring at 60 ℃ for 60 min and then cooled to room temperature. A vitamin C tablet was dipped into the solution to form a thin layer of zein coating. In addition to the zein solution, pectin solution was prepared for making the zein-pectin coated dosage form. It was formed by slowly adding 200 mg of pectin in water with vigorous stirring. 10 mg of glycerol was added to the pectin solution. The solution was heated with gentle stirring at 60 ℃ for 60 min and then cooled to room temperature. The pectin solution was degassed by ultrasound. Zein-pectin coated dosage forms were made by coating a vitamin C tablet. First, the tablet was dipped into the zein solution for 5 s. Excess solution was drained off. When the zein coating was dried at room temperature, the tablet was then dipped into the pectin solution, and immediately dipped into the zein solution to form a zein-pectin layer. The zein-pectin coating was allowed to dry and coating in pectin and zein solutions was repeated.

2.6 Thickness measurement

The thickness of the films prepared in Section 2.3 was measured at five random points using a micrometer (Mitutoyo Corp., Japan) at an accuracy of 0.01 mm.

2.7 Mechanical properties

Ultimate tensile strength and elongation at break of the zein films were measured using a digital dynamometer (Handpi, China) in accordance with the ASTM D882 standard method. They were cut into dumb-bell shape with overall width (b_2) of 25 mm and length (L_2) of 100 mm.

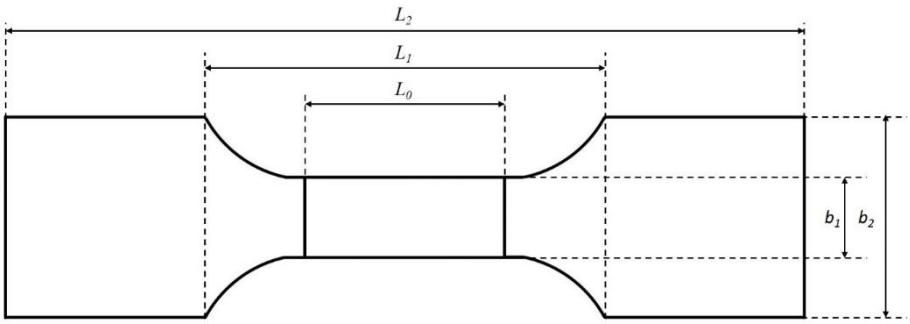


Figure 2.1 Shape and dimensions of the zein films.

Samples were mounted on the grip at separation (L_1) of 50 mm. The gauge length (L_0) was 25 mm and the width of the narrow portion was 10 mm. The samples were stretched at 50 mm/min. The tensile strength and percent elongation at break were calculated as follows:

$$TS = \frac{F}{A} \; and \; \%E = \Delta L / L_0$$

where *F* was the maximum load, *A* was the cross sectional area of the film, ΔL was the extended length, and L_0 was the initial gauge length.

2.8 Water vapor permeability

A zein solution was prepared as described in Section 2.3 and then 3.5 g of the solutions were poured on a petri dish. After drying at room temperature, zein films were detached and conditioned at 50% RH for at least 48 hours. WVP was measured gravimetrically in accordance with the ASTM standard method using a Payne permeability cup (Elcometer 5100, Belgium) which had an exposed area of 10 cm^2. The cup was filled 3 ml of distilled water. The film samples were attached to the cup with clamping at the ledge. The assembly was weighed using an analytical balance at an accuracy of 0.0001 g. It was placed in a desiccator with silica gel under controlled atmosphere at 23 ℃ and 50% RH for 24 hours. The temperature and humidity was monitored using a thermohygrometer. WVP was calculated using the following equation:

$$WVP = \frac{\Delta m \times T}{A \times t \times \Delta p}$$

where Δm was the weight change of the assembly, *T* was the thickness of the film, *A* was the exposed area, *t* was the time during weight change, and Δp was the vapor pressure difference.

2.9 Water solubility test

The zein films were conditioned in a desiccator containing silica gel, at 23 ℃ and 50% RH for at least 48 hours before the measurement. About 20 mg of film samples were weighed using an analytical balance at an accuracy of 0.0001 g. The samples were soaked in 50 ml of distilled water with gentle stirring at 37 ℃ for 24 hours. They were then blot dried using filter papers. The samples were heated at 105 ℃ for 2 hours and then cooled to room temperature in the desiccator. After cooling, the samples were weighed accurately. Water content and water solubility were determined as follows:

$$\text{Water content of the capsule}(\%) = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100\%$$

$$\text{Water solubility}\ (\%) = \frac{\text{Final dry weight} - \text{initial dry weight}}{\text{initial dry weight}} \times 100\%$$

2.10 Swelling test

The swelling behaviors of zein, pectin, and zein-pectin films were evaluated by incubating the film samples in 200 ml of phosphate buffer solution of pH 7.4 at 37 ℃ for 6 hours. A portion of the zein solution prepared in Section 2.5 was poured onto a petri dish to form a zein film. The zein-pectin film was prepared by mixing 1:1 ratio of the zein and pectin solutions thoroughly and dried on a petri dish. Because pectin film dissolved readily in water, 50 mM calcium chloride cross-linked pectin film was

prepared to reduce the solubility. 20 mg of the films was weighed for the measurement. At certain time intervals, samples were blot dried and weighed. The swelling index (SI) was calculated as follows:

$$SI = (m_t - m_0)/m_0$$

where m_t was the weight of the film at time point *t*, and m_0 was the initial weight of the samples.

2.11 Fourier transform infrared spectroscopy

The changes in secondary structure of the plasticized zein films were analyzed using FTIR-ATR. The samples were prepared the same as Section 2.3. The chemical structures of zein, pectin, and zein-pectin films were also characterized. The films were prepared similarly as Section 2.10, except the pectin film was not added with calcium chloride. The three solutions were poured in separate petri dishes. Film samples were obtained after drying. The samples were analyzed using an FTIR Spectrometer.

2.12 Scanning electron microscopic studies

The film samples prepared in Section 2.3, and the samples coated with a zein layer and one or two zein-pectin layer(s) on vitamin C tablets, were examined using

SEM. Before SEM, samples were freeze-fractured using liquid nitrogen. Sample cross sections were coated with gold (300 Å) to increase conductivity, using an Edwards S150B sputter coater. The sample cross sections were imaged with SEM.

2.13 Differential scanning calorimetric studies

T_g of the film samples prepared in Section 2.3 was analyzed using DSC. About 5 mg of the samples were accurately weighed in a crucible and covered with a cap. An empty crucible was used as a reference. The samples were scanned from 30 to 220 ℃ at a rate of 5 ℃/min. T_g was determined from the thermograms by the change of heat flow at the mid-point of onset and end temperatures using a software Netzsch Proteus Thermal Analysis.

2.14 Dissolution studies of zein capsule

The *in vitro* release studies were performed by incubating the oleic acid-plasticized zein capsules in simulated gastrointestinal fluids at 37 ℃. 20 mg of vitamin C powder was encapsulated in the capsules. The capsules were enclosed in capsule sinkers to avoid floating and sticking to the vessel. The method was modified from Kshirsagar[78] and Barker[32] and their coworkers. Four different release media were prepared, named as simulated gastric fluid (SGF), simulated intestinal fluids (SIF),

which represented stomach, duodenum, jejunum and ileum, respectively. The compositions of the media were prepared as follows:

SGF: 2 g/L sodium chloride, 7 g/L pepsin, pH 1.2.

SIF-duodenum: 6.8 g/L sodium phosphate monobasic, 3.6 g/L pancreatin, pH 6.5.

SIF-jejunum: 6.8 g/L sodium phosphate monobasic, 2.8 g/L pancreatin, pH 6.8.

SIF-ileum: 6.8 g/L sodium phosphate monobasic, pH 7.2.

pH of the media was adjusted using 1 M of hydrochloric acid and 1 M of sodium hydroxide solutions. Continuous release studies were carried out by incubating the capsules in 100 ml of each medium, SGF for 2 hours, SIF-duodenum for 1 hour, SIF-jejunum for 2 hours, and SIF-ileum for 1 hour. At certain time intervals, 1 ml of the media was withdrawn for vitamin C determination and 1 ml of the fresh media was added for replacement.

2.15 Dissolution studies of zein-pectin coated dosage forms

Zein-pectin coated dosage forms encapsulating vitamin C tablets were examined for dissolution test using SGF and SIF. In addition, the colon-specific zein-pectin coated dosage forms were examined in simulated colonic fluid (SCF). Each solution

was prepared to a total volume of 100 ml. The samples were enclosed in a dialysis tubing (MWCO 10,000, packed wet) with 5 ml of the gastrointestinal solution with enzymes. SGF was composed of 0.2% (w/v) sodium chloride and pH was adjusted to 1.2 by 1 M of hydrochloric acid, followed by the addition of 0.7% (w/v) pepsin. SIF was prepared with 0.68% (w/v) sodium phosphate and pH was adjusted to 7.4 by 1 M of sodium hydroxide, followed by 1% (w/v) pancreatin. SCF was composed of 1.3% (w/v) potassium phosphate, 0.13% (w/v) disodium hydrogen phosphate, and 0.3% (v/v) pectinase. The solutions were incubated at 37 ℃ under gentle stirring. The dosage forms were first incubated in the SGF for 2 hours. At certain time intervals, 1 ml of the solution was withdrawn for vitamin C content determination and 1 ml of the fresh media without enzyme was added to the solutions. After incubation in SGF, the samples were transferred to SIF. The duration of the dissolution tests in SIF and SCF were 4 hours and 18 hours respectively.

2.16 Determination of vitamin C concentration

HPLC was used to determine the vitamin C content in the simulated gastrointestinal fluids. The method was modified from Hu and coworkers.[79] The withdrawn solutions from the *in vitro* release were filtered with 0.45 μm syringe filter, and a portion of 20 μl was injected into a HPLC-DAD system equipped with a RP-

column (LiChrosorb® 250 mm × 4.6 mm, 5 µm particle size). Milli-Q water/methanol (80:20) was utilized as the mobile phase with a flow rate of 1 ml/min at room temperature. Ascorbic acid concentration was determined at a wavelength of 245 nm.

Chapter 3 Characterization and Dissolution Studies of Zein Capsule

3.1 Introduction

In this study, a delayed release capsule was developed using zein as the major biopolymer. Zein is a unique material which has excellent film forming property, and its film is grease and solvent resistant. Taking advantages of these characteristics, the utilization of zein raised at a time. However, due to the major limitations of zein, i.e. high extraction cost and the brittleness of the film, zein has not been widely used. Studies has been extensively conducted to improve the mechanical properties of zein using various kinds of plasticizers and crosslinking agents. The use of plasticizers reduces the brittleness and increases the ductility of the zein films.

With the improved mechanical properties, a delayed release capsule should be resistant to gastric digestion. Zein exhibits certain resistance to pepsin digestion in gastric fluid, but it is susceptible to pancreatin digestion.[66] However, the effect of plasticizers on the release of zein capsule is unknown. In this project, the mechanical properties of the zein films and the dissolution studies of the zein capsules are discussed in the following sections.

3.2 Results and discussions

3.2.1 Mechanical properties of zein films

Mechanical property describes the relationship between the stress and strain of a material under an applied force. Tensile strength and elongation were the major values determined to evaluate the brittleness and ductility of the material. One of the factors that limits the utilization of zein is the brittleness. Brittle materials usually have high tensile strength but low elongation. Zein films absorbed little energy and broke without plastic deformation. In order to make zein more favorable for industrial applications, it would be important to improve the mechanical properties. The currently used capsule material is mainly made from gelatin. Table 3.1 shows the mechanical properties of the gelatin and zein films, and they were plasticized at the same level with glycerol. Compared to the gelatin film, the zein film had considerably lower tensile strength and elongation. The poor mechanical properties of the zein films caused failure in capsule manufacture and were unsatisfactory for commercial use. Studies demonstrates that the use of plasticizers enhance the elongation of the zein films, however the tensile strength reduces. Crosslinking agent may be added to improve the tensile strength, and one of the mostly investigated nontoxic agents is citric acid. But no evidence has shown that citric acid has impacts on the mechanical strength of the zein films.

Material	Tensile strength (MPa)	Elongation (%)
Gelatin	48.04 ± 3.59	47.71 ± 4.78
Zein	26.49 ± 0.47	9.83 ± 1.35

Table 3.1 Tensile strength and elongation at break of gelatin and zein films. The films were plasticized with glycerol at the level of 15% weight of the materials.

In this study, three types of plasticizers, i.e. oleic acid, PEG and glycerol, were studied. They were added to the zein solutions at different weight ratio to zein. The zein films were cut into dump-bell shape and conditioned for tensile measurement. Their effects on the ultimate tensile strength and elongation at break of the zein films were investigated and presented in Table 3.2 to Table 3.4, showing the results at different plasticizing levels of the three plasticizers. Unplasticized zein films acted as a control sample, having tensile strength of 27.54 ± 1.70 MPa and elongation at break of 7.92 ± 0.88%. In general, addition of the plasticizers resulted in reduction of tensile strength but improvement of elongation. Oleic acid-plasticized zein films had the lowest tensile strength and elongation, while PEG- or glycerol-plasticized films had comparable tensile strength and improved elongation.

Oleic acid is a fatty acid which can be found in animal and vegetable origins. The

use of oleic acid as a plasticizer has been studied in various materials, such as gelatin, zein, sodium caseinate and chitosan-lithium acetate.[80-83] The water solubility and compatibility of oleic acid and the materials are the major concerns. Because oleic acid is water insoluble, it is not compatible with gelatin, resulting in decrease in the mechanical strength of the films. Whereas, a number of studies found improvement of mechanical strength of oleic acid-plasticized zein films. In this project, oleic acid was added to plasticize zein films at various concentrations. It was found that there was significant reduction in tensile strength and elongation for all plasticizing levels. Oleic acid-plasticized zein films at the levels of 7 and 10% exhibited similar tensile strength at 11.54 and 11.11 MPa. At the levels of 15 to 20%, tensile strength of the zein films increased to about 16 MPa, in which 20% plasticizing level showed the highest tensile strength of 18.68 MPa. However, further addition of oleic acid from 30 to 70% caused considerable decrease in tensile strength. For elongation, an increasing trend was observed when a higher amount of oleic acid was added to the zein films. Low plasticizing levels caused significant decrease in elongation to about 3-4% compared to the control sample. These results generally agree with those presented by other studies. Lai and coworkers[84] suggested a layered structure parallel to the surface of the zein films was formed after drying from the casting process. Oleic acid adsorbed in between the layered hydrophilic surfaces of the zein films and led to dispersion of the

zein molecules. Such adsorption increased the separation of the protein chains and hence improved the flexibility of the films. They also demonstrated that different preparation process of the zein films could result in changed structures having different tensile properties.

Plasticizing level (weight percent to zein)	Tensile strength (MPa)	Elongation (%)
7	11.54 ± 0.62	3.03 ± 0.14
10	11.11 ± 0.50	3.41 ± 0.21
15	16.13 ± 1.05	3.95 ± 0.28
20	18.68 ± 2.17	4.50 ± 0.36
30	16.44 ± 0.53	4.15 ± 0.24
50	14.99 ± 0.72	5.64 ± 0.07
70	10.83 ± 0.46	6.91 ± 0.60

Table 3.2 Tensile strength and elongation at break of oleic acid-plasticized zein films.

PEG is a widely used as a medical excipient. It is water soluble and has a low toxicity. Plasticization using PEG in films was also studied often using low-molecular-weight PEGs, such as 300, 400 and 1000. They were found to be effective in plasticizing various film-forming materials, like chitosan, poly(lactic acid) and zein.[85-87] In this project, high-molecular-weight PEG having 15-20 kDa was added to the zein films to study its plasticizing effect. From Table 3.3, the PEG-plasticized zein films had generally higher tensile strength and elongation than the oleic acid-plasticized zein films. Low tensile strength and elongation at 7% plasticizing levels were observed. Further increase in PEG concentration to 10% resulted in maximum elevated tensile strength and elongation compared to other concentrations. The plasticizing effects of PEG at 15, 20 and 30% on the zein films were comparable. Poor mechanical properties were found at 50 and 70% as a result of limited capacity of the zein films. Tillekeratne and coworkers had investigated the plasticizing effect of PEG 400 and 1000 on the zein films. They found that PEG 1000 was more effective than PEG 400.[88] High molecular weight molecules enhanced the separation between protein layers to a greater extent, resulting in a more flexibility protein network. Segregation of the zein films was observed at high PEG plasticizing levels, which was the same as that obtained from the study done by Baiardo and coworkers using poly(lactic acid).[87]

Plasticizing level (weight percent to zein)	Tensile strength (MPa)	Elongation (%)
7	14.65 ± 0.64	4.47 ± 0.04
10	29.38 ± 0.82	11.24 ± 0.33
15	26.17 ± 1.50	7.05 ± 0.33
20	28.27 ± 1.13	8.65 ± 0.24
30	27.80 ± 0.82	8.11 ± 0.44
50	17.04 ± 0.47	6.93 ± 0.28
70	12.29 ± 1.51	5.51 ± 0.36

Table 3.3 Tensile strength and elongation at break of PEG-plasticized zein films.

Glycerol is another common plasticizer for many materials especially the water soluble polymers, such as gelatin and starch. It is water soluble and hydroscopic. Glycerol-plasticized zein films had also been extensively studied with certain degree of improvement of the mechanical strength. As shown in Table 3.4, unlike oleic acid and PEG, glycerol-plasticized zein films had the highest tensile strength at a low plasticizing level. However, the tensile strength decreased as the glycerol concentration was increased from 32.02 to 3.24 MPa at 7 to 70% plasticizing levels. The elongation of the zein films was improved by plasticization at 10 and 15% weight ratio of glycerol to zein. Gao and coworkers[89] described the change of mechanical behavior of the glycerol-plasticized kafirin films at different concentrations of glycerol. At low concentrations of glycerol, it led to water binding into the protein's specific parts as 'structural water'. The plasticization effect of glycerol was mainly due to the protein-glycerol interactions, while protein-protein, and glycerol-glycerol interactions were also found. Similar to the PEG-plasticized zein films, segregation of the glycerol-plasticized films at higher glycerol concentrations was observed. This may be due to domination of glycerol-glycerol interactions.

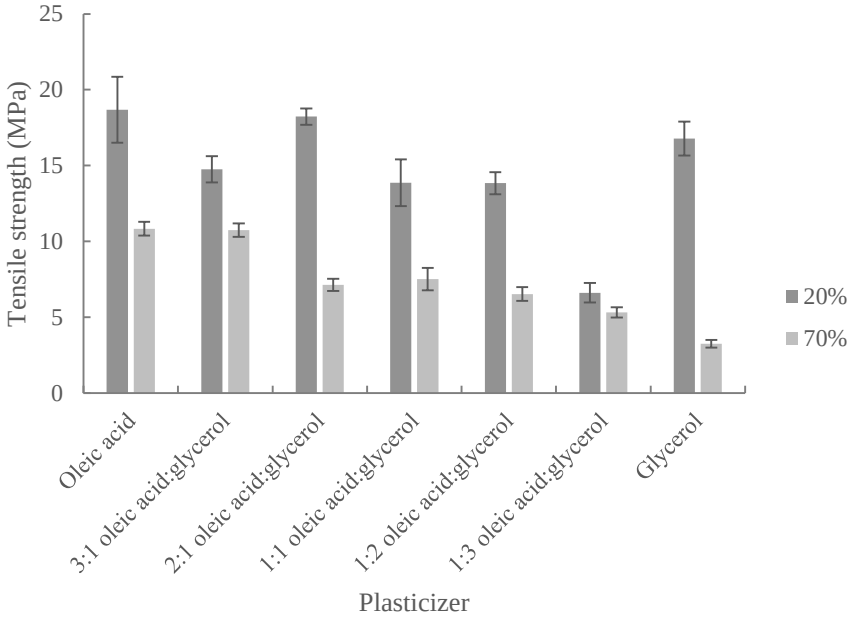
Plasticizing level (weight percent to zein)	Tensile strength (MPa)	Elongation (%)
7	32.02 ± 0.61	5.90 ± 0.91
10	26.58 ± 1.04	9.89 ± 0.60
15	26.49 ± 0.47	9.83 ± 1.35
20	16.77 ± 1.12	4.99 ± 0.14
30	9.63 ± 0.41	3.54 ± 0.15
50	6.88 ± 0.49	3.88 ± 0.66
70	3.24 ± 0.25	3.49 ± 0.44

Table 3.4 Tensile strength and elongation at break of glycerol-plasticized zein films.

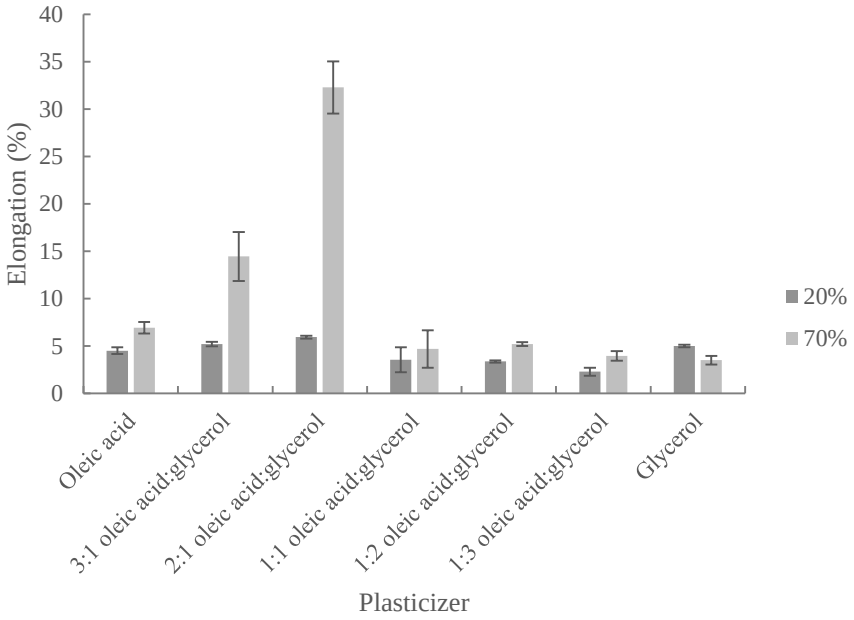
Oleic acid and PEG were classified as the primary plasticizers of zein films because they were effective in reducing the brittleness of the films when used alone. For glycerol, it was considered as a secondary plasticizer. Its plasticizing effect was not as satisfactory as the primary plasticizers, but combining itself with a primary plasticizer may result in greater improvement of the mechanical properties of the zein films. Thus, oleic acid and PEG were combined with glycerol respectively to investigate whether they have synergistic effect.

Oleic acid and glycerol were added to plasticize the zein films at different ratios and plasticizing levels. The mechanical properties of the plasticized zein films were shown in Figure 3.1. The total plasticizing levels were 20 and 70%. They demonstrated different trends in the tensile strength and elongation of the plasticized zein films. At 20%, oleic acid, 2:1 oleic acid-to-glycerol or glycerol-plasticized zein films showed the highest tensile strength, while tensile strength of 1:3 oleic acid-to-glycerol zein films was the lowest. All 20% plasticized zein films had elongation below or about 5%, in which 2:1 oleic acid-to-glycerol exhibited the highest value of 5.92%. This shows that the combination of oleic acid and glycerol did not have significant improvement of the mechanical strength of the plasticized zein films. Synergistic effect was not observed from plasticizing level of 20%. Whereas 70% plasticizing

level may show a more obvious change in the mechanical properties. At 70%, the tensile strength of the plasticized zein films for all combinations was smaller than those of 20%. The higher the amount of glycerol, the lower the tensile strength of the zein films was. At combination of lower glycerol concentrations, elongation increased with the glycerol content reaching maximum at 2:1 oleic acid-to-glycerol of 32.27%. Further increase in the glycerol concentrations in the combination resulted in significant reduction of elongation to about 3-5%. The results were generally similar to those presented by Xu and coworkers.[90] They prepared a zein solution by dissolving 1 g of zein in 10 ml of 80% ethanol and zein films were formed by casting. Zein has an amphophilic structure. And oleic acid is hydrophobic, while glycerol is hydrophilic. The combination of oleic acid and glycerol balanced the polarity of zein, in which oleic acid interacted with zein molecules by hydrophobic interactions and glycerol formed hydrogen bonds with the molecules. Thus, they formed a promising network with improved mechanical properties. However, Xu and coworkers proposed that synergistic effect was observed at 3:1 oleic acid-to-glycerol level. In this work, 2:1 oleic acid-to-glycerol plasticized zein films at 70% total plasticizing level had the better mechanical properties regarding the ductility.



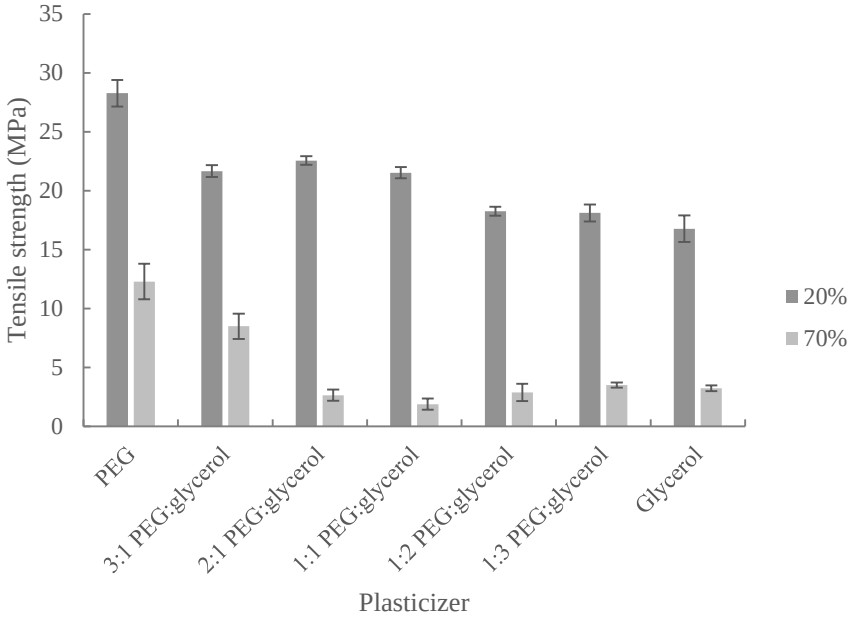
(a)



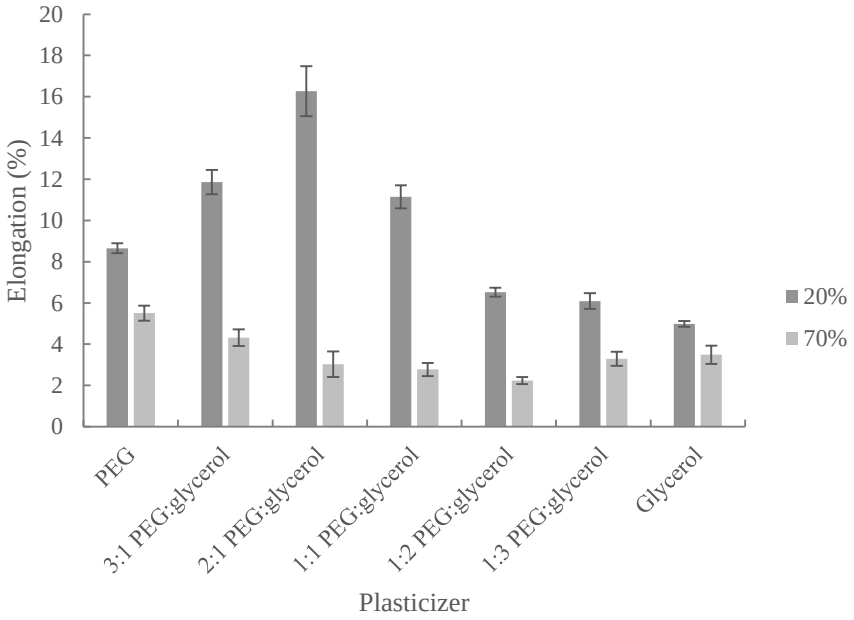
(b)

Figure 3.1 (a) Tensile strength and (b) elongation at break of zein films plasticized with different ratios of oleic acid to glycerol at total plasticizing levels of 20 and 70%, respectively.

The effect of combining PEG and glycerol was also investigated. Figure 3.2 shows the tensile strength and elongation at break of the 20 and 70% PEG-glycerol-plasticized zein films. At both levels, decrease in tensile strength was observed after the addition of glycerol to the zein films. Whereas, the elongation of the films varied in different ways for the two levels. In general, 20% plasticizing level of the zein films had better mechanical properties than 70%. The tensile strength of the 20% plasticized zein films ranged from 18.26-22.56 MPa. Similar to the oleic acid-glycerol-plasticized films, PEG-glycerol-plasticized films had the highest elongation at 2:1 PEG-glycerol combination. Comparable tensile strength and elongation were observed for 3:1 and 1:1 PEG-glycerol combinations. A higher portion of glycerol resulted in smaller tensile strength and significant reduction of elongation. The zein films at 70% plasticizing levels generally showed poorer mechanical behaviors. Parris and coworkers[63] prepared a zein solution by dissolving 1 g of zein in 10 ml of 80% ethanol and the PEG and glycerol plasticized films were formed by casting on petri dishes. They had obtained similar results showing synergistic effect of the combined use of PEG and glycerol on the zein films.



(a)



(b)

Figure 3.2 (a) Tensile strength and (b) elongation at break of zein films plasticized with different ratios of PEG to glycerol at total plasticizing levels of 20 and 70%, respectively.

3.2.2 Water vapor permeability

WVP is defined as the rate of water vapor transmitted through the unit area of a material of unit thickness induced by unit vapor pressure difference between two specific surfaces, under specific temperature and humidity conditions. In this project, WVP was determined using gravimetric technique and the water method was selected. Water was added into the Payne permeability cup and the zein film was mounted on it. Weight loss of the assembly under a controlled atmosphere was measured, and the WVP was calculated with known temperatures and humidity conditions.

Plasticization of the zein films varied the WVP, depending on the type of plasticizers added. Table 3.5 shows the effect of different type of plasticizers on the WVP of the zein films. The unplasticized zein films had certain degree of WVP due to the amphiphilic and hydroscopic nature of zein. At the plasticizing level of 70%, significant difference between the plasticizers was obtained. Oleic acid-plasticized zein films reduced the WVP compared to the control sample, while PEG and glycerol plasticization caused two-fold and four-fold increase. The difference was due to the hydrophilicity of the plasticizers. The addition of oleic acid to the zein films caused reduction of WVP from 3.65 to 2.40 $\times 10^{-10} gm^{-1}s^{-1}Pa^{-1}$ because oleic acid is a hydrophobic compound and it is water insoluble. Oleic acid interacted with the layered

structure of zein films. Oleic acid suppressed the absorption of water and penetration of water through the zein films. And it was possible that the hydrophobic interaction between oleic acid and zein blocked the passage of water of the zein films. This would be favorable for various applications with water barrier properties. In contrast to the oleic acid, PEG and glycerol have higher polarity which make them hydrophilic and water soluble. When they were incorporated into the zein films, water may be absorbed and penetrated through the films easily. Compared to glycerol, PEG has a bulky structure and less hydroxyl groups. Thus, it had smaller value of WVP.

Because oleic acid and glycerol had opposite effect on the WVP, the effect of combined use of these plasticizers on the WVP was also evaluated. Unplasticized (control) and plasticized zein films with different ratio of oleic acid to glycerol were prepared and then conditioned before the measurement. Figure 3.3 shows the WVP of the films. Compared to the control sample, the addition of oleic acid resulted in slight decrease in the WVP. When the ratio of oleic acid to glycerol reduced from 3:1 to 1:3, there was a gradual and almost linear increasing trend of the WVP. And the zein films plasticized with glycerol alone had significantly higher WVP than others. This shows that the hydrophobicity of oleic acid not only suppressed the WVP of zein, but also the WVP of glycerol.

Plasticizer	WVP ($10^{-10}gm^{-1}s^{-1}Pa^{-1}$)
Control	3.65 ± 0.07
Oleic acid	2.40 ± 0.22
PEG	7.32 ± 0.28
Glycerol	13.6 ± 0.23

Table 3.5 WVP of zein films added with different plasticizer at the level 70% to the weight of zein.

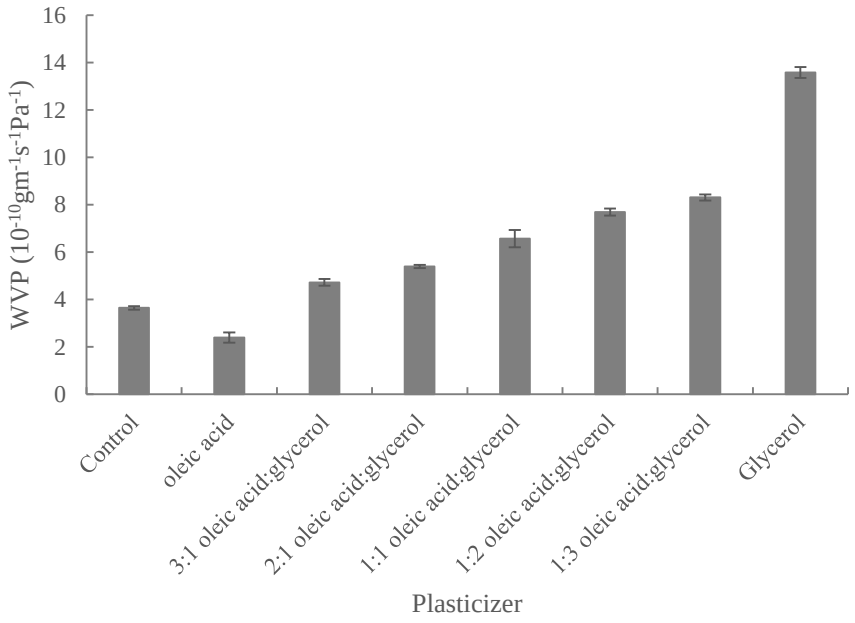


Figure 3.3 WVP of zein films at different ratio of oleic acid to glycerol. Total plasticizer content was 70% to the weight of zein.

3.2.3 Water solubility test

Water solubility test was conducted by immersing the zein films in distilled water with stirring under a controlled temperature in a certain period of time. Weight loss of the zein films was obtained and this value implied the amount of water soluble portion in the film had dissolved in the liquid medium. As demonstrated above, different types of plasticizers had different water permeability behavior and this may be related to the water solubility of the plasticized zein films.

Table 3.6 presented the water solubility of the corresponding plasticized zein films. The control sample had a water solubility of 7.76% after incubation in distilled water at 37℃ for 24 hours. Although zein contains mostly hydrophobic amino acid residues, it has the amphiphilic and hydroscopic properties. Therefore, it showed certain degree of water solubility. As oleic acid was incorporated into the zein films, significant reduction of water solubility was observed. It formed hydrophobic interactions with zein molecules which prevented structural and physical destruction when the plasticized films were immersed in water. Such hydrophobic interactions may reduce the binding of water to the structure and thus, water solubility was lower. All other plasticizers had an increased water solubility. Compared to the control, PEG caused a two-fold increase while glycerol had almost three-fold increase in water

solubility. These two plasticizers were hydrophilic and water soluble. When the plasticized zein films were immersed in the water, the hydrophilic interactions between the plasticizers and water molecules destroyed the structure of the zein films. The value of water solubility may account for the amount of the free or dissociated plasticizers dissolved and the soluble zein molecules. For oleic acid-glycerol-plasticized zein films, increased water solubility of 11.51% was obtained. Similarly, oleic acid showed certain degree of suppression of water solubility, and this was probably owing to the hydrophobic interactions between oleic acid and zein, and glycerol. Whereas, the water solubility of PEG-glycerol-plasticized zein films was comparable with that of PEG. This was possibly because PEG was dominant in the plasticizer blend and the hydrophilic interactions between PEG and glycerol reduced water binding to the molecules.

Plasticizer	Water solubility (%)
Control	7.76 ± 2.20
Oleic acid	1.52 ± 2.20
PEG	16.20 ± 2.16
Glycerol	19.03 ± 3.46
Oleic acid-glycerol	11.51 ± 1.95
PEG-glycerol	16.35 ± 1.79

Table 3.6 Water solubility of the plasticized zein films incubated in distilled water at 37℃ for 24 hours. The total plasticizing levels were 20% weight ratio to zein. Both oleic acid-to-glycerol and PEG-to-glycerol-plasticized at the ratio of 2:1.

3.2.4 Fourier transform infrared spectroscopy

FTIR is a classical method for structural analysis, and the chemical composition and architecture of small molecules can be identified. Information of protein structures and molecular reactions can also be obtained from the FTIR spectrum. It measures the change of vibrational state of molecules excited by the absorption of infrared radiation, implying the strength and polarity of the vibrating bonds.[91] Chemical structures of proteins cannot be fully interpreted from FTIR spectrum, but the changes of the chemical bonding can be detected. Moreover, vibrational spectroscopy allows direct interpretation of the strength of hydrogen bonds by the frequency of stretching vibrations. The band position, intensity, and width, provide conformational information.

In this project, FTIR-ATR was used to evaluate the structural change of the zein films. The spectra of the plasticized films were compared and analyzed. From the spectrum of the control, three major distinct bands were obtained at 3289, 1651 and 1540 cm^{-1}. The broad bands at around 3200 cm^{-1} represented O–H stretching of hydrogen bonds. And the bands at 1500-1700 cm^{-1} represented the two characteristic amide I and amide II groups of protein molecules. Amide I and II were the C=O stretching and C–N stretching of the amide, respectively. Hydrogen bonding and

interactions between the plasticizers and zein molecules can be interpreted from the spectra.

Compared to the control, the band of oleic acid at around 3200 cm^{-1} had no difference in intensity and no shifting was observed. This implied that oleic acid and zein molecules may not interact with each other by hydrogen bonding. Whereas, the bands of oleic acid at 1500-1700 cm^{-1} had a different intensity with the control. The band at 1651 cm^{-1} changed from 64 to 60%, and the band at 1540 cm^{-1} changed from 69 to 72%. The increased absorption at 1651 cm^{-1} may be due to the hydrophobic interactions between oleic acid and zein molecules altering the structure of the amide I band. For oleic acid-glycerol and glycerol-plasticized zein films, they had very similar spectra. The bands at 3289 cm^{-1} changed to 82 from 86%, which implied that the addition of glycerol formed hydrogen bonds with zein molecules. And the band at 1651 and 1540 cm^{-1} had transmittance of 55 and 62% respectively. This indicated that glycerol interacted with zein molecules causing conformation change.

For PEG-plasticized zein films, significant change of the intensity from 86 to 78% at band 3200 cm^{-1} was observed, indicating the formation of hydrogen bonds between PEG and the zein molecules. At the band 1651 cm^{-1}, the absorption change

considerably from 64 to 48%. The band at 1540 cm^{-1} also shows change of absorption from 69 to 58%. For PEG-glycerol-plasticized zein films, the bands at 3289 cm^{-1} changed to 83 from 86%. Changes were also observed for bands at 1500-1700 cm^{-1}, which were from 64 to 57% for the band at 1651 cm^{-1} and from 69 to 64% for the band at 1540 cm^{-1}.

From the above results obtained, no obvious change in the bands of the plasticized zein films compared to the unplasticized films. The vibrations of amide I and II bands were sensitive to the conformational change of the protein structure, but the change in the side chain usually did not affect the bands. Plasticizers interacted with the side chains of the zein molecules may not be reflected from the spectra. And the change in the secondary structure of the backbone was unlikely to occur. This implied that hydrophilic and hydrophobic interactions between the plasticizer and the zein molecules were more likely to happen.

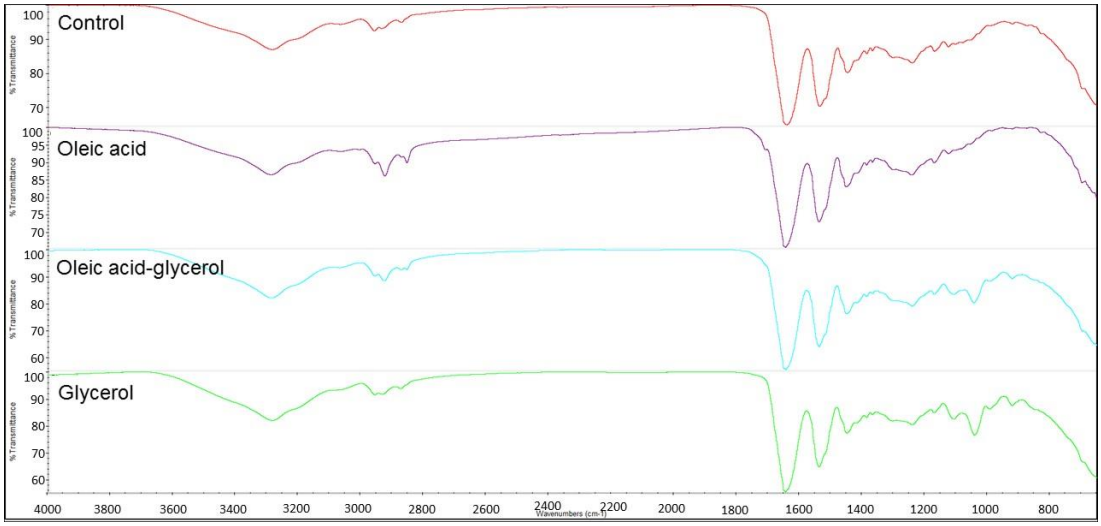


Figure 3.4 FTIR spectra of the oleic acid-plasticized zein films. The total plasticizing levels were 20% weight ratio to zein. Oleic acid-to-glycerol-plasticized at the ratio of 2:1.

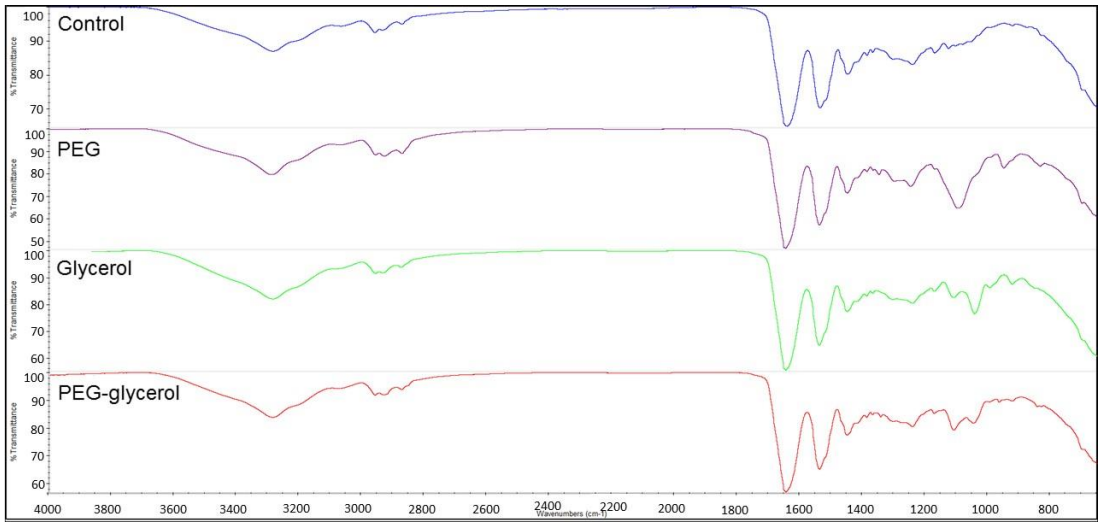


Figure 3.5 FTIR spectra of the PEG-plasticized zein films. The total plasticizing levels were 20% weight ratio to zein. PEG-to-glycerol-plasticized at the ratio of 2:1.

3.2.5 Scanning electron microscopic studies

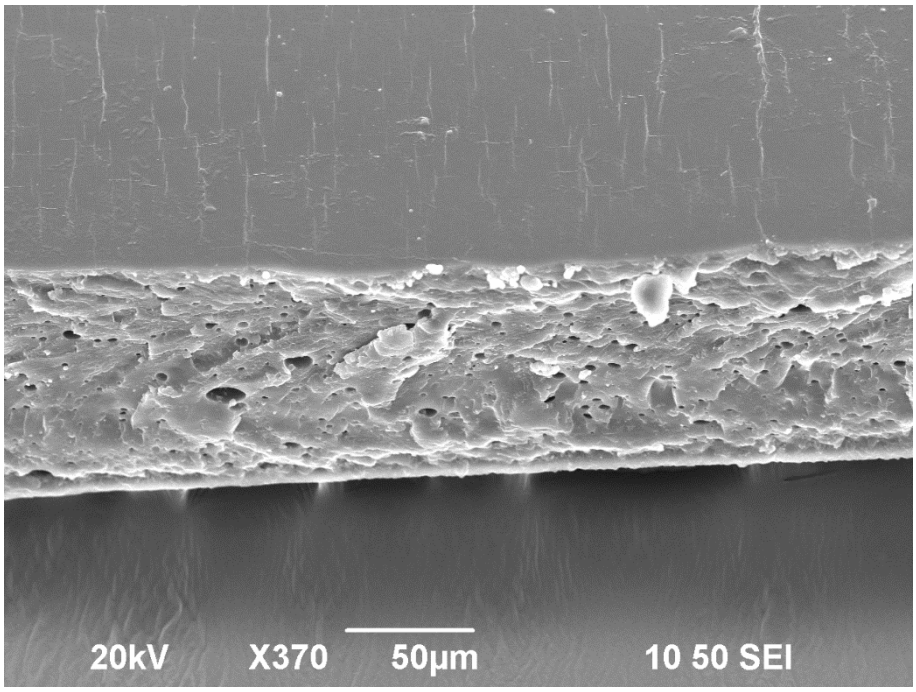
SEM examines the physical and supramolecular morphology of material surface. In this project, the cross sections and the surface morphology of the zein films were evaluated using SEM and the samples were freeze-fractured. In order to increase the electric conductivity, they were coated with gold. Figure 3.6 to Figure 3.11 presents the SEM images of the plasticized film samples and their characteristic structures will be discussed.

A control sample, which was an unplasticized zein film, had a relatively smooth surface with a few tiny pores. The formation of the pores may be due to loss of water during conditioning under 50% RH and 25 ℃. For oleic acid-plasticized zein films, more and larger pores were found in the cross section and the surface of the films. When oleic acid was added to the zein solution, it formed a clear yellowish solution and showed good compatibility in the solution. It was because oleic acid was highly soluble in alcohol solution. However, when the films were completely dried, ethanol was evaporated. Oleic acid is not water soluble, therefore losing the compatibility in the zein films. Although oleic acid formed hydrophobic interactions with the layered zein molecules, free oleic acid may be found. It may be escaped from the films resulting in an oily surface and the formation of the pores. For PEG-plasticized films,

they shows similar morphology as the control sample having a number of small holes and relatively smoother surface. Unlike oleic acid, PEG is water and ethanol soluble. It showed good compatibility in the zein solution as well as in the films. As ethanol evaporated, the zein solution become more hydrophilic. Because of the presence of hydroxyl groups in PEG and its high molecular weight, it may be able to form hydrophilic and hydrophobic interactions with zein molecules. Thus PEG was able to form a tighter and better network with the protein chains than oleic acid. For glycerol, many small holes were found on the cross section and the surface of the films. The presence of glycerol caused structurally binding of water molecules in the zein films. When the films were conditioned at 50% RH and 25℃, pores may be formed as a result of losing the bound water from the structure. Combining glycerol with oleic acid improved film smoothness and fewer pores were formed. This shows the synergistic effect of the blended plasticizers on improving the structural morphology of the films. The PEG-glycerol plasticized films were structurally similar to which plasticized by PEG, but having more pores on the surface.

It was found that the physical structure examined under SEM may be related to the mechanical strength of the films. The tensile strength of the films at the plasticizing levels of 20% was PEG>Control>PEG-glycerol>oleic acid>oleic-glycerol>glycerol,

and the elongation was PEG-glycerol>PEG>Control>oleic acid-glycerol>glycerol>oleic acid. The control, PEG and PEG-glycerol-plasticized films had similar surface morphology, in which PEG-glycerol-plasticized films had more pores. And similar morphology was found in oleic acid, glycerol and oleic acid-glycerol-plasticized films, while glycerol-plasticized films had the most porous structure. It was proposed that the more porous structure would result in weaker tensile strength while there was no relationship between the structure of the films and their ductility.



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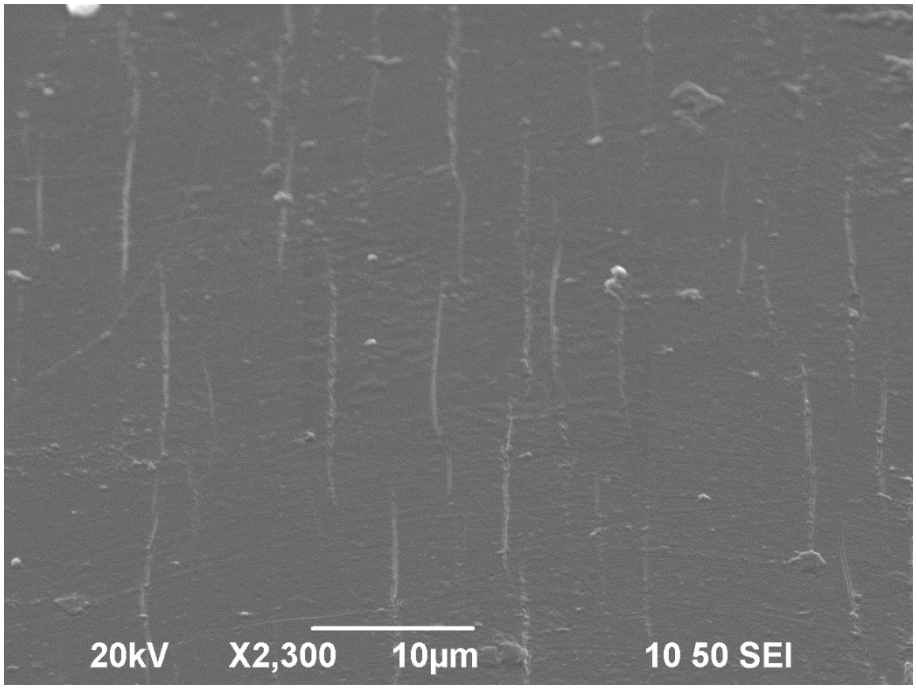
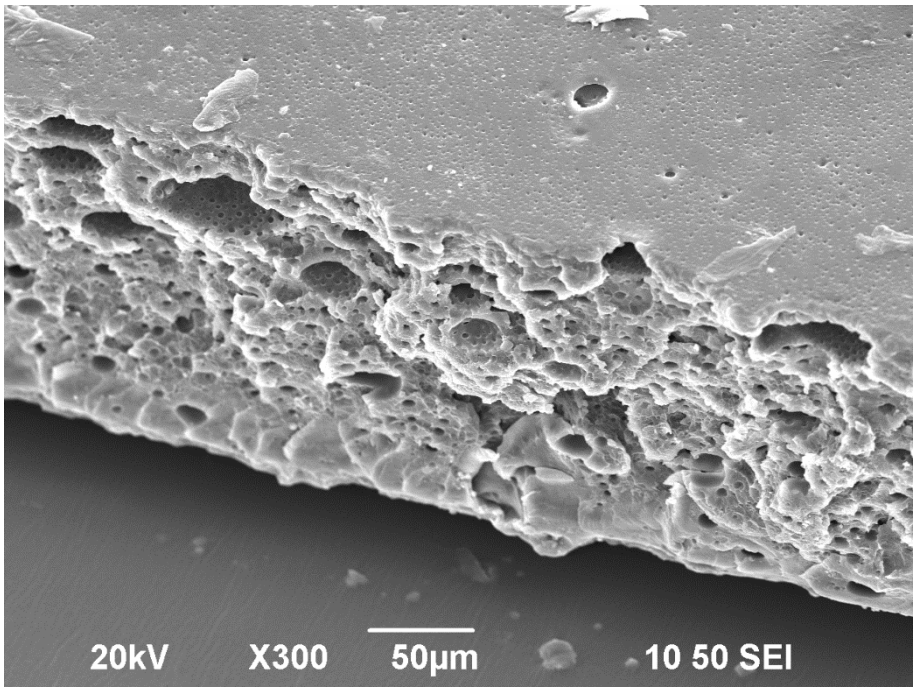


(b)

Figure 3.6 SEM images of (a) the cross section and (b) the surface of the control sample.



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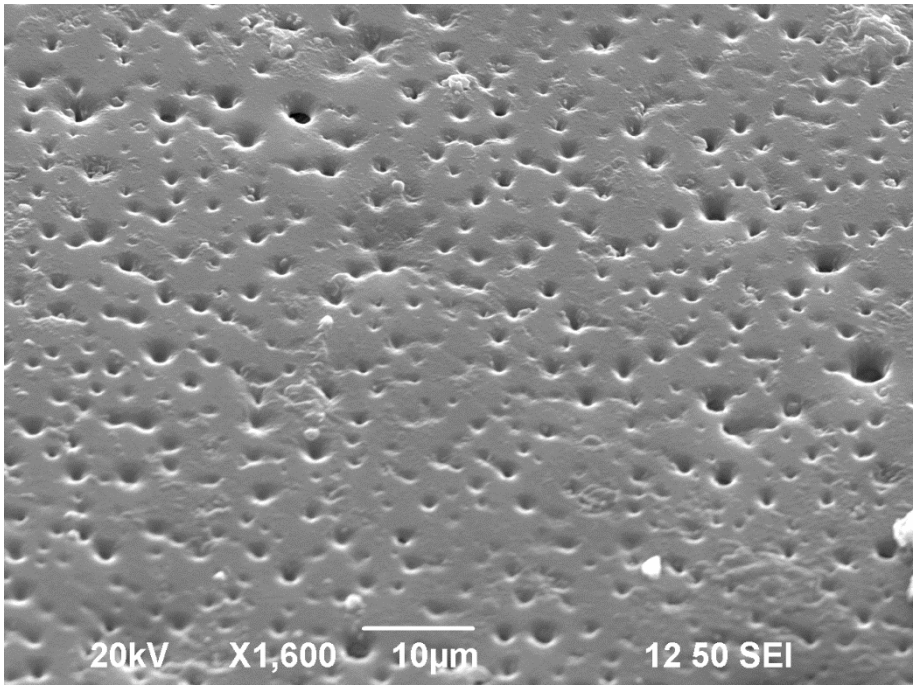
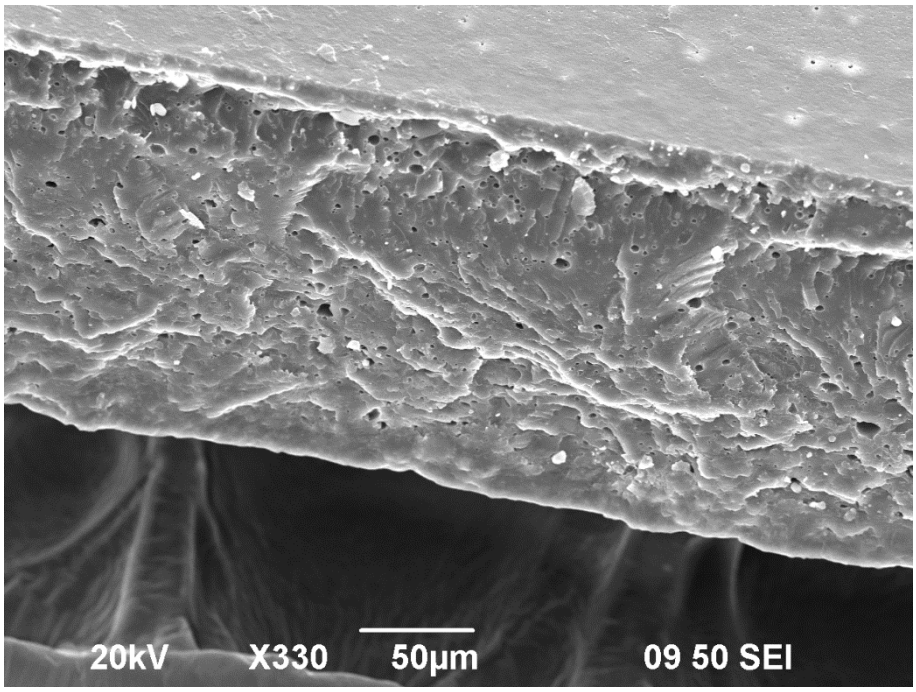


(b)

Figure 3.7 SEM images of (a) the cross section and (b) the surface of the oleic acid-plasticized films. The total plasticizing level was 20%.



(a)

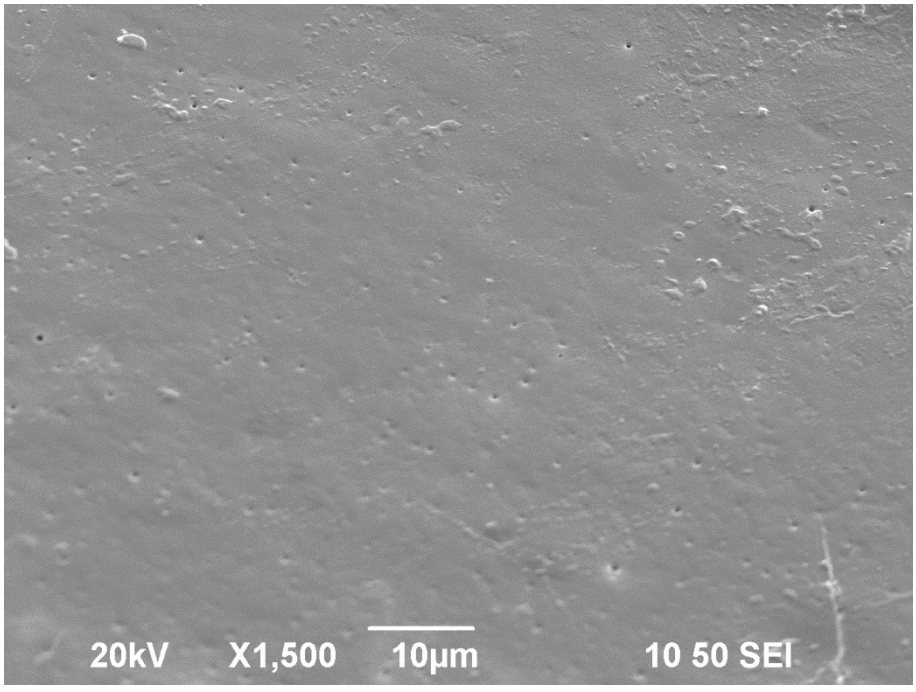


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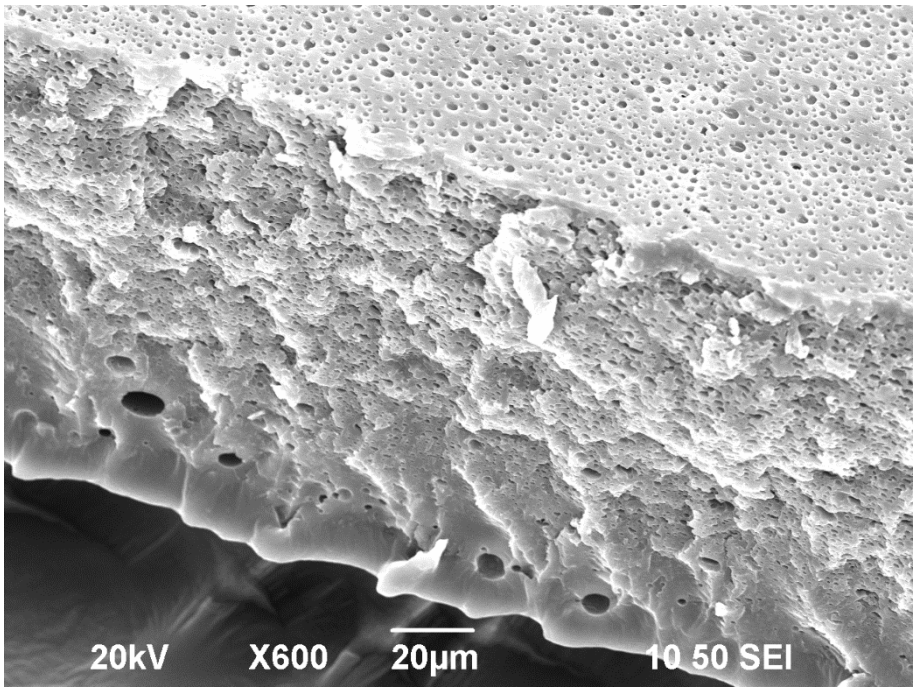
Figure 3.8 SEM images of (a) the cross section and (b) the surface of the PEG-plasticized films. The total plasticizing level was 20%.



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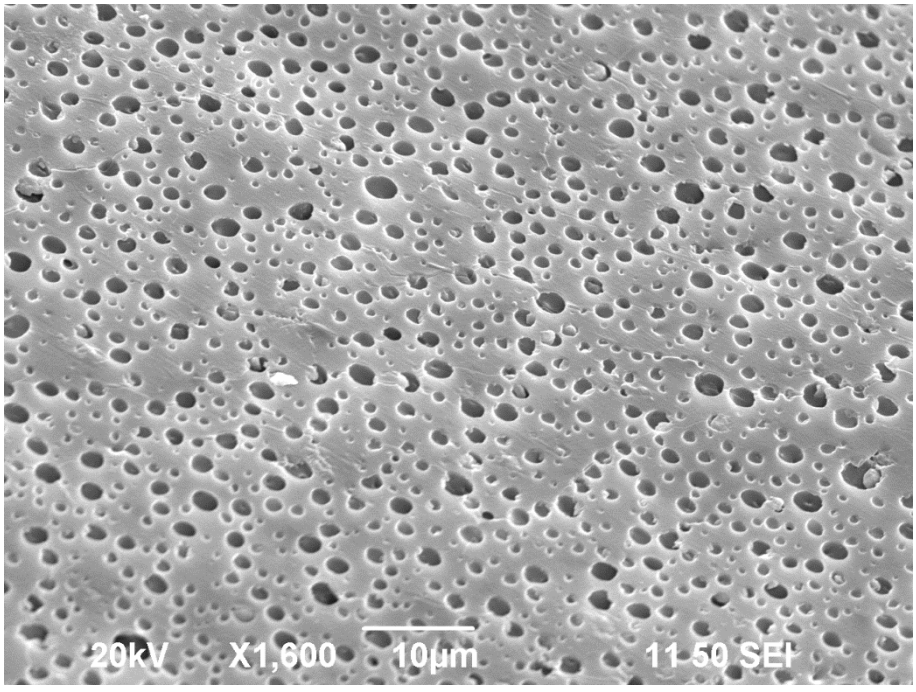
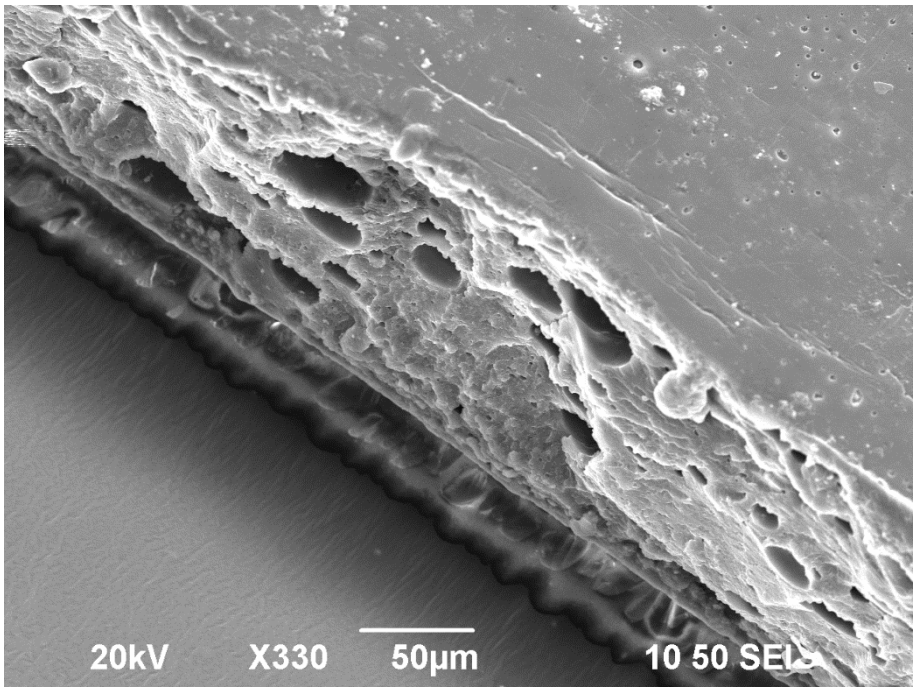


(b)

Figure 3.9 SEM images of (a) the cross section and (b) the surface of the glycerol-plasticized films. The total plasticizing level was 20%.



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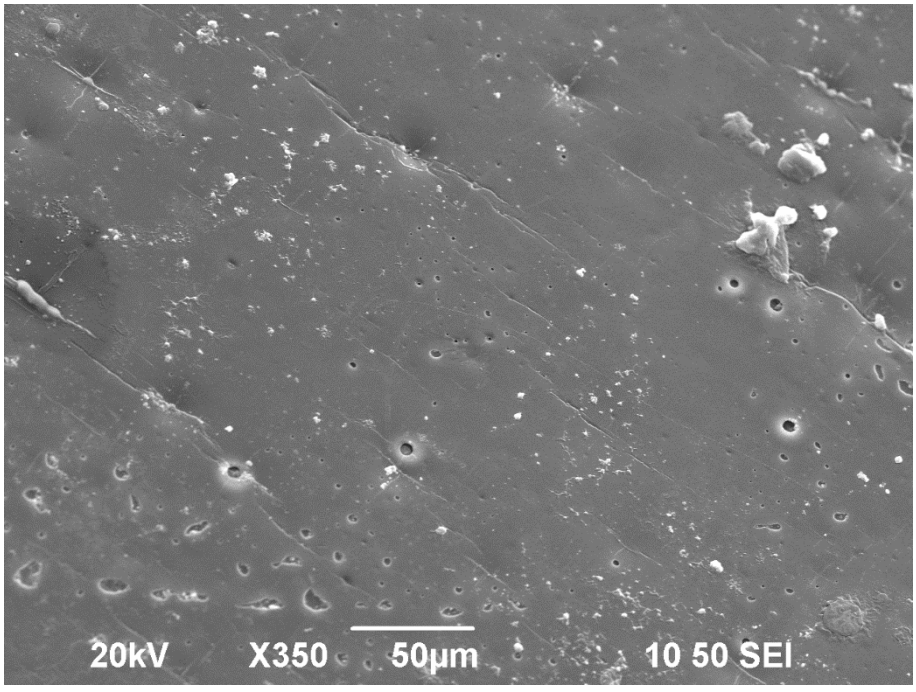
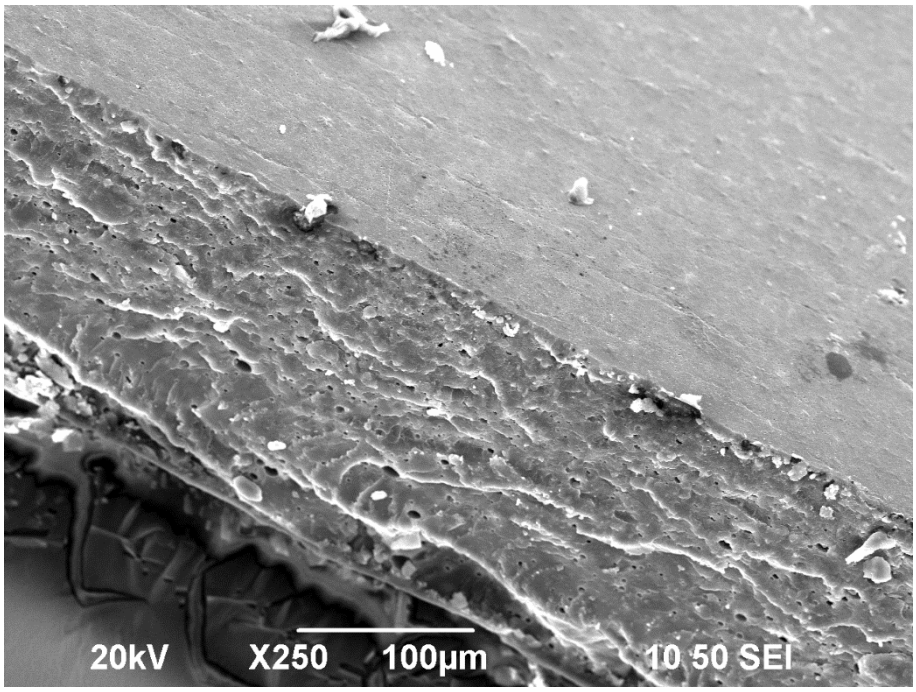


(b)

Figure 3.10 SEM images of (a) the cross section and (b) the surface of the oleic acid-glycerol-plasticized films. The total plasticizing level was 20%.



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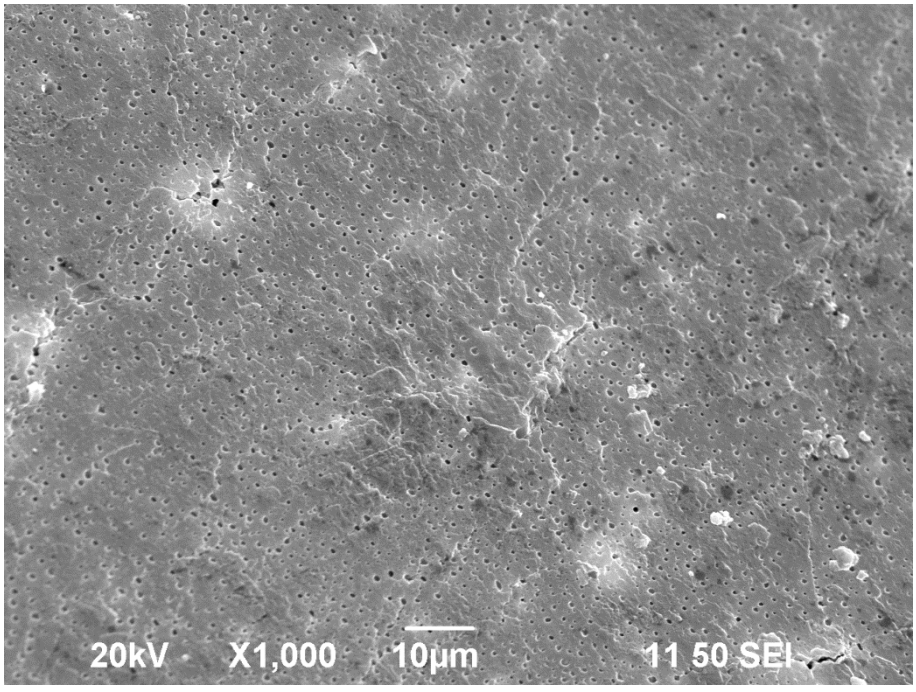

(b)

Figure 3.11 SEM images of (a) the cross section and (b) the surface of the PEG-glycerol-plasticized films. The total plasticizing level was 20%.

3.2.6 Differential scanning calorimetric studies

Glass transition is defined as the change of a hard and brittle material to a rubbery solid. DSC is used to determine the temperature that indicates the change of heat capacity when glass transition occurs. In this project, T_g of the plasticized zein films was measured to evaluate the effect of the plasticizers on the structure of the zein films as well as the interactions between the plasticizers and the zein molecules.

Because water is an effective plasticizer of the zein films, the water contents of the films were determined. It was found that all types of films had similar level of water contents which should not impose a significant impact on the value of T_g. The control which was the unplasticized zein films had the highest value of T_g at 124.6℃. The value was similar to that stated in the literatures.[84, 90] Reduction of T_g was found in all plasticized films due to the hydrophobic and hydrophilic interactions between the plasticizers and the zein structure because the glass transition is mainly governed by intermolecular interactions.[92] The decrease in T_g of oleic acid-plasticized films was due to the hydrophobic interactions between oleic acid and the zein layered structures and adsorption of oleic acid in the layers, leading to reduction of friction. For glycerol, hydrogen bonds formation with the zein molecules separate the protein chains in a great extent and consequently a significant reduction of T_g was observed. Similar to

the plasticizing effects of glycerol, PEG formed hydrogen bonds with zein molecules and the bulky structure of PEG provided more space between the molecules, so the T_g of the plasticized film decreased. In contrast to the study conducted by Xu and coworkers,[90] plasticizer blend of oleic acid and glycerol did not show a further reduction of the T_g. But this is consistent with the results obtained in the mechanical properties measurement. There was actually no obvious improvement of the tensile strength and elongation of the oleic acid-glycerol-plasticized zein films at the plasticizing level of 20%. Whereas the PEG-glycerol-plasticized zein films have a further reduction of the value of T_g. And this is also consistent with the measurement of the mechanical strength. Therefore, synergistic effect of PEG and glycerol was significant at the level of 20%. This may be due to the extended network formation between PEG, glycerol and the zein molecules by their intermolecular forces. The bulky structure of high molecular weight PEG and small molecule glycerol penetrated into the zein layers, creating large free volume between the protein chains.

Plasticizer	Water content (%)	Glass transition temperature (℃)
Control	5.97	124.6
Oleic acid	5.86	119.4
PEG	6.06	104.0
Glycerol	6.54	94.7
Oleic acid-glycerol	5.98	95.5
PEG-glycerol	6.14	91.6

Table 3.7 T_g of the zein films added with different plasticizers as determined by DSC. The total plasticizing levels of the films were 20%. Both oleic acid-to-glycerol and PEG-to-glycerol plasticized at the ratio of 2:1.

3.2.7 Disintegration test

Delayed release capsule is resistant to gastric digestion and deliver the encapsulated drug to the small intestine. In this project, disintegration test was performed by incubating the capsules in 50 ml of different media under 37 ℃ for 2 hours. Four types of capsules plasticized by oleic acid, PEG, oleic acid-glycerol and PEG-glycerol respectively, were selected with satisfactory mechanical properties. Distilled water, SGF and SIF (duodenum) were prepared to evaluate the effect of these media on the degradation of the plasticized capsules by visual observation. Results of the disintegration test were shown in Table 3.8.

In the disintegration test, the control capsule was not available for measurement because it was brittle and broke easily. In distilled water, all capsules remained intact. Higher water solubility of PEG, oleic acid-glycerol and PEG-glycerol zein films did not show significant impact on the disintegration of the capsule. After incubation in SGF, only oleic acid-plasticized zein capsule showed intactness. For oleic acid-glycerol-plasticized capsule, partial disintegration was observed at the shoulder of the capsule because it was the thinnest part. Disintegration of PEG and PEG-glycerol capsules were observed in SGF and fragments were found. It may be due to their high water solubility and this led to higher accessibility to enzymes. Thus, PEG and

glycerol may not be suitable plasticizers for the controlled release zein capsules. During incubation in SIF (duodenum), all capsules were disintegrated because zein was susceptible to digestion of pancreatin.

Plasticized capsule	Distilled water	SGF	SIF (duodenum)
Oleic acid	Intact	Intact	Disintegrated
PEG	Intact	Disintegrated	Disintegrated
Oleic acid-glycerol	Intact	Partially disintegrated	Disintegrated
PEG-glycerol	Intact	Disintegrated	Disintegrated

Table 3.8 Disintegration test of the plasticized capsule. Oleic acid and 2:1 oleic acid-to-glycerol capsules were plasticized at the level of 70%. PEG and 2:1 PEG-to-glycerol capsule were plasticized at the level of 20%.

3.2.8 Physical parameters of zein capsules

The zein capsule was trimmed to regular length. Figure 3.12 presents the picture of the two-piece hard capsule of size #0 made from oleic acid-plasticized zein. Table 3.9 shows the physical parameters of the zein capsule.

Figure 3.12 A two-piece zein hard capsule of size #0 plasticized with oleic acid.

Parameter	Plasticized zein capsule
Body length (mm)	17.73 ± 0.06
Cap length (mm)	10.44 ± 0.25
Body thickness (mm)	0.12 ± 0.01
Cap thickness (mm)	0.12 ± 0.01
Body outer diameter (mm)	7.30 ± 0.01
Cap outer diameter (mm)	7.65 ± 0.01
Average weight (mg)	74.4

Table 3.9 Physical parameters of plasticized zein capsules.

3.2.9 Dissolution studies

Dissolution studies of the delayed release capsule mimic the *in vivo* conditions of human by *in vitro* test. Based on the current understanding about the gastrointestinal physiology, the fluid composition, transit time and pH of the different segments in GI tract are simulated. In this project, four simulated gastrointestinal fluids were prepared, and they were SGF, SIF (duodenum, jejunum and ileum). An oleic acid-plasticized zein capsule was selected for the cumulative release measurement. Vitamin C powder was encapsulated into the capsule. The capsule was enclosed in a capsule sinker which prevented floating to ensure the reproducibility of the test. It was incubated in the SGF for 2 hours, followed by SIF-duodenum for 1 hour, SIF-jejunum for 2 hours and SIF-ileum for 1 hour. The release profile of the capsule is presented in Figure 3.13 and analyzed as follows.

During the first 2 hour-release test in SGF, no release was detected at time 0 and 30 min. The release started after 30 min incubation and increased to 2.4% at 1 hour and finally reached 6.4% at 2 hours. The zein capsule was not soluble in water and it had certain resistant to gastric digestion of pepsin. Therefore, little release was obtained in SGF owing to the limited digestion by pepsin. Then the capsule was transferred to SIF-duodenum. Only slight elevation of the release was observed at 2.5

hours. The release rate increased and 15.7% total release was attained after 3 hours. Among the three segments of the small intestine, duodenum contained the most enzyme but had lower pH. The increased release rate showed the action of pancreatin on the digestion of the zein capsule, resulting in more release of vitamin C. The release was continued in SIF-jejunum and steady release rate was shown. During the following 2 hour-incubation, the release increased from 15.7 to 90.7% and the rate became slower starting from 4.5 hours. Fragments of the capsules were found in the medium which indicated disintegration of the zein capsule as a result of protease digestion. Such a high release rate was due to the large amount of pancreatin in jejunum. Finally, the zein capsule was transferred to SIF-ileum which did not contain any enzymes. The release rate decreased significantly compared to that in SIF-jejunum and 93.8% total release was found after incubating for 6 hours in the media. Ileum was a site of absorption and where digestion of the zein capsules was not obvious. This showed that the structure of the zein capsules was stable in the absence of enzymes. Whereas most of the vitamin C had been release in SIF-jejunum.

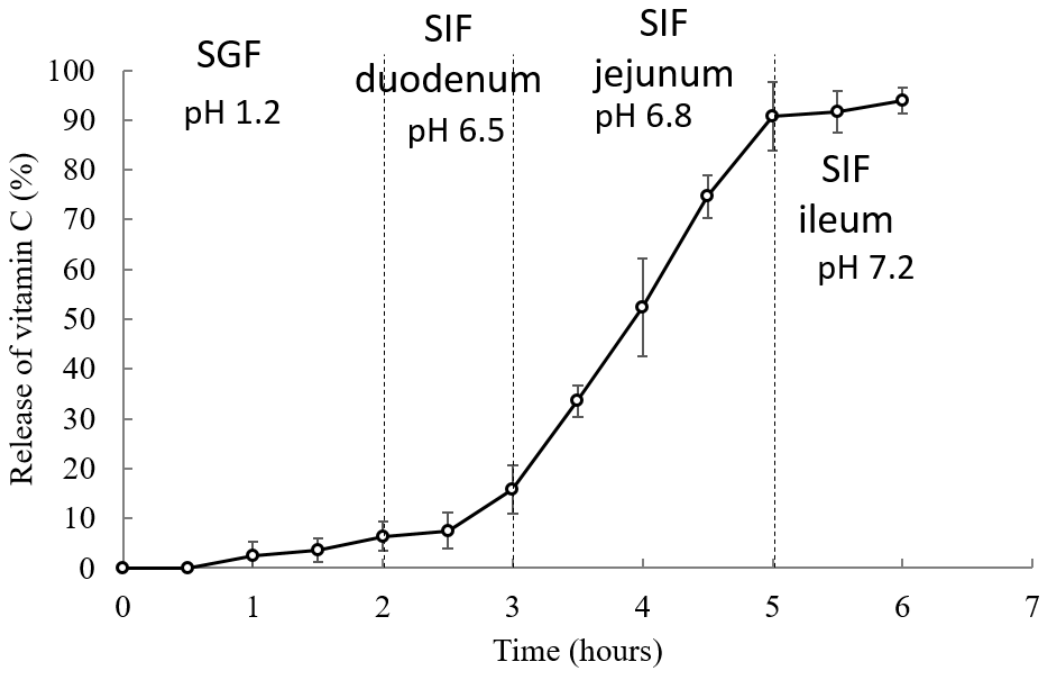

Figure 3.13 Release profile of the oleic acid-plasticized zein capsules. The capsule was incubated in the release media SGF for 2 hours, SIF-duodenum for 1 hour, SIF-jejunum for 2 hours and SIF-ileum for 1 hour.

3.3 Conclusions

Plasticization of the zein films using oleic acid, PEG, glycerol and their combinations had improved the mechanical properties. No observable change to the secondary structure of the zein films was found, but hydrophilic and hydrophobic interactions were likely to occur between the plasticizers and the zein molecules. Different plasticizers formed different interactions with the zein molecules resulting in characteristics change in the tensile properties, water permeability, supramolecular structure and glass transition. Due to its hydrophobicity, oleic acid improved the water barrier property of the films. For a high molecular weight PEG, significant enhancement of the tensile properties of the films was observed. Although glycerol was not a good plasticizer when used alone, synergistic effects were found if it was combined with the two primary plasticizers. Owing to the high water solubility of PEG and glycerol, the plasticized zein film disintegrated rapidly in SGF and SIF. Thus, only oleic acid was the suitable plasticizer for the formation of controlled release zein capsule. The dissolution results shows that zein capsule has a great potential to be developed as a delayed-release dosage form.

Chapter 4 Characterization and Dissolution Studies of Zein-Pectin Coated Dosage Form

4.1 Introduction

In Chapter 3, it was found that zein capsule had controlled release property and delayed drug release in the gastric fluid. However, in order to develop a colon-specific drug delivery system, using zein alone may not be possible to achieve it. As found by Liu and coworkers,[75] zein and pectin could be made as a compatible drug delivery system and compensate for the weakness of each other by forming hydrogel beads. Such a system may also be applicable to solid dosage form.

In this chapter, the *in vitro* performance of the zein-pectin coated dosage forms was evaluated. Their structural characteristics and physiochemical properties were analyzed using SEM, FTIR-ATR and the swelling test. The rationale of the design of the functional dosage forms made from zein-pectin was also discussed.

4.2 Results and discussions

4.2.1 Fourier transform infrared spectroscopy

The chemical interactions between zein and pectin were analyzed using FTIR-ATR. Zein, pectin, and zein-pectin films were prepared. Figure 4.1 shows the spectra of the films with characteristic peaks labeled. The bands at around 3200 cm^{-1} represented O–H stretching of hydrogen bonds. In a zein-pectin mixture, O–H stretching band became more intense and shifted from 3289 to 3285 cm^{-1}. It was proposed that amide groups of zein and hydroxyl groups of pectin were interacted to form strong hydrogen bonds.

In zein and zein-pectin, there were two characteristic bands at 1500-1700 cm^{-1} which represented the amide I and amide II groups. Amide I band represented C=O stretching of the amide in protein, while amide II band represented C–N stretching of the amide. There was a shifting of amide I band from 1651 to 1647 cm^{-1} while shifting of amide II band was not significant. The shifting was probably due to the hydrogen bonds formation between zein and pectin. No observable shifting found in amide II band may be because no significant change in the secondary structure of the zein molecules. This was because amide bands were sensitive to the change of the secondary structure, but not the interactions of the side chains.[91]

In pectin and zein-pectin, there were strong bands at 1000-1200 cm^{-1}. They were the C–O stretching of alcohols, carboxylic acids, and esters. The band shifted from 1020 to 1039 cm^{-1} indicating structural changes of the functional groups. However, zein did not have such bands. Thus, it was believed that zein-pectin having the combined characteristic IR bands of zein and pectin was due to the formation of zein-pectin complex.

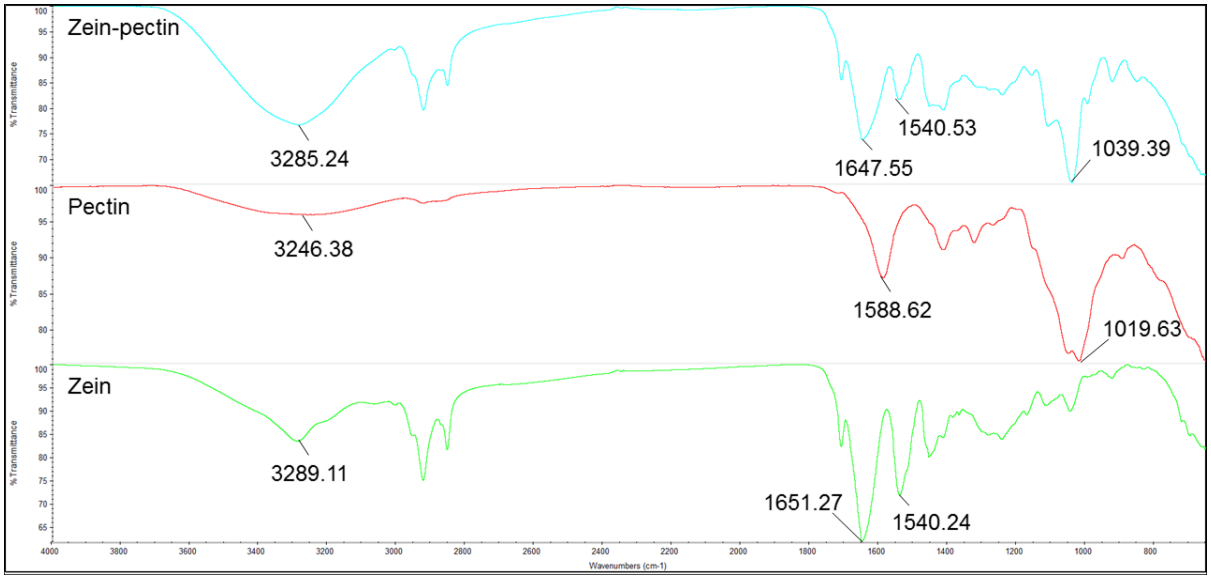

Figure 4.1 FTIR-ATR spectra of zein, pectin and zein-pectin films.

4.2.2 Scanning electron microscopic studies

Zein-pectin coated dosage form for colon-specific drug delivery was prepared by the dip coating technique. The cross-section of the zein-pectin capsule was examined by SEM. Figure 4.2 shows the SEM images of the zein-pectin dosage form for colon-specific drug delivery. They were taken from the freeze-fracture surfaces of the coated vitamin C tablets. Layers were formed on tablets. The left hand side was the outermost layer of the capsule. The outer and middle layers had similar thickness while the inner layer was thinner. The outer and inner layers showed rough surfaces with no pore and crack, whereas the middle layer had a porous structure.

Another sample was prepared by dipping a vitamin C tablet in the zein solution and dried. It was then dipped in the pectin solution, followed by the zein solution. The cross-section of the sample was also examined in SEM. Figure 4.3 shows the morphology of zein-pectin film made from a zein layer and a zein-pectin layer. Images were taken from the freeze-fracture surfaces of the coated vitamin C tablets. Two layers were observed on tablets. The outer layer was thicker than the inner layer. In general, both layers showed smooth surface with no pore or crack. At the edge of the outer layer, rough surface was observed.

The outer and middle layers of zein-pectin capsule in Figure 4.2 show similar thickness. It was proposed that both layers are composed of zein and pectin mixture. While both layers are prepared in the same way, they should have the same morphology. However, the middle layer has a porous structure while the outer layer does not show any pores. Figure 4.3 shows a zein-pectin layer, which has similar thickness as that of the zein-pectin layers of the zein-pectin capsule in Figure 4.2. The zein-pectin layer in Figure 4.3 shows similar morphology as the outer layer of the zein-pectin capsule.

Based on the structures of the capsules shown in the SEM images, we proposed a formation mechanism of the zein-pectin dosage form, which can be explained with the aid of the schematic diagram in Figure 4.4. There are three layers in the capsule, i.e. zein (A), middle zein-pectin (B) and outer zein-pectin (C). In general, the boundary between layers A and B (zein boundary) and the boundary between layers B and C (zein-pectin boundary) can cause the porous structure of the middle zein-pectin layer.

Vitamin C tablet was dipped into the zein solution and then dried. A thin zein layer (A) was formed on the tablet surface. After that, the zein-coated tablet was dipped into the pectin solution, followed by the zein solution. Because the zein layer

was completely dried before dipping into the pectin solution and zein was insoluble in water, it did not mix with the pectin solution. After coating with pectin solution, zein solution was immediately coated on the tablet. Pectin would mix with the zein solution to form a zein-pectin layer (B). There was a zein boundary separating these two layers. From the SEM images, pores were observed in layer B. Larger and more pores were found near layer A. Zein was hydrophobic in nature while pectin was hydrophilic. Also, zein layer A and pectin solution were of two different phases. Therefore, zein-pectin layer B did not adhere well to the zein boundary. However, the one-layer zein-pectin coating showed smooth and rough structure without pore. This was also observed in the outer zein-pectin layer (C) for the zein-pectin capsule. It was suggested that the dipping action of layer C and the zein-pectin boundary between layers B and C affected the structure of zein-pectin mixture. Layers B and C were alike in chemical structures, resulting in stronger adhesion at zein-pectin boundary than zein boundary. Thus, layer B between zein boundary and zein-pectin boundary formed a characteristic structure.

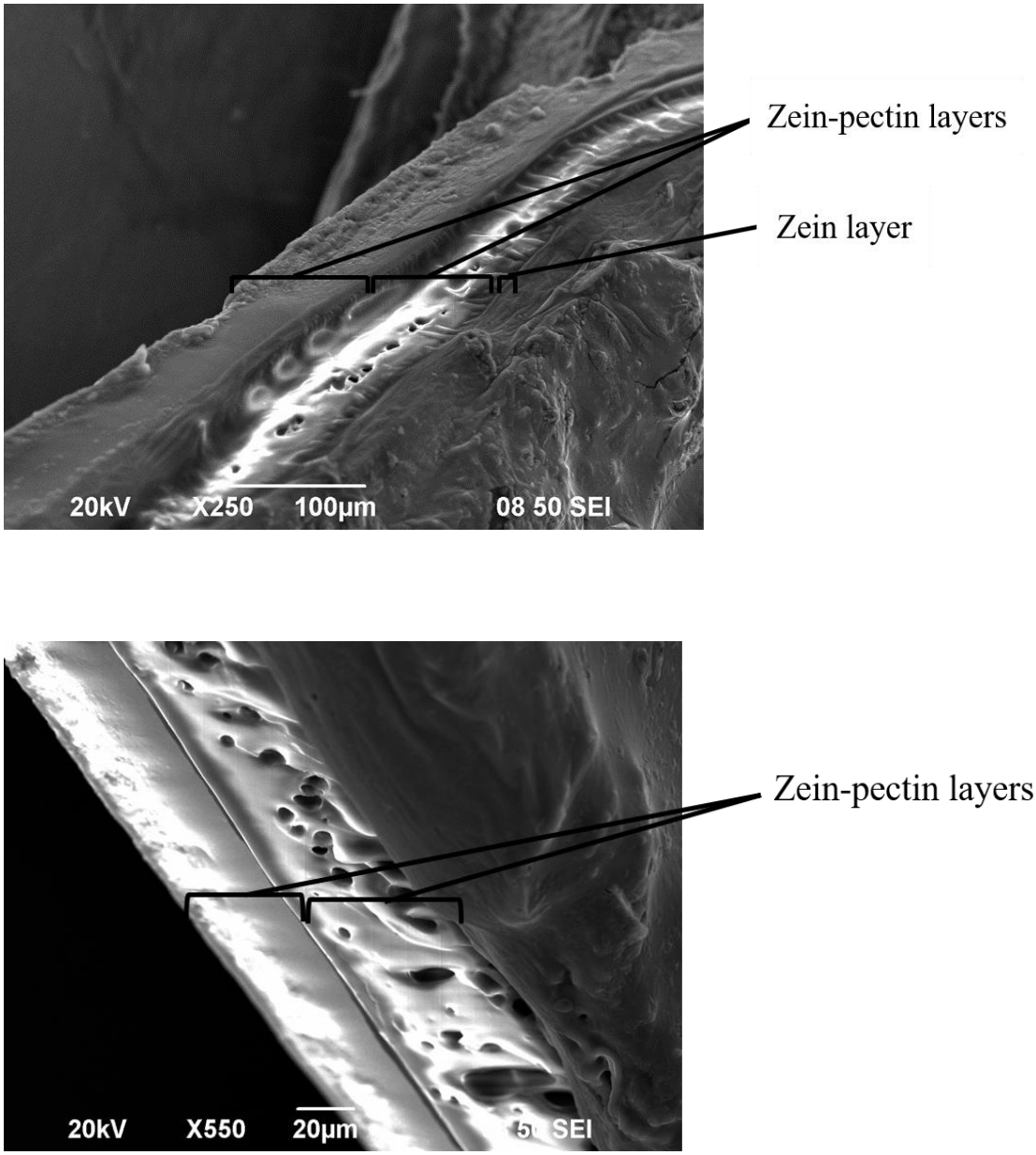

Figure 4.2 SEM images of the cross section of the zein-pectin coated dosage form.

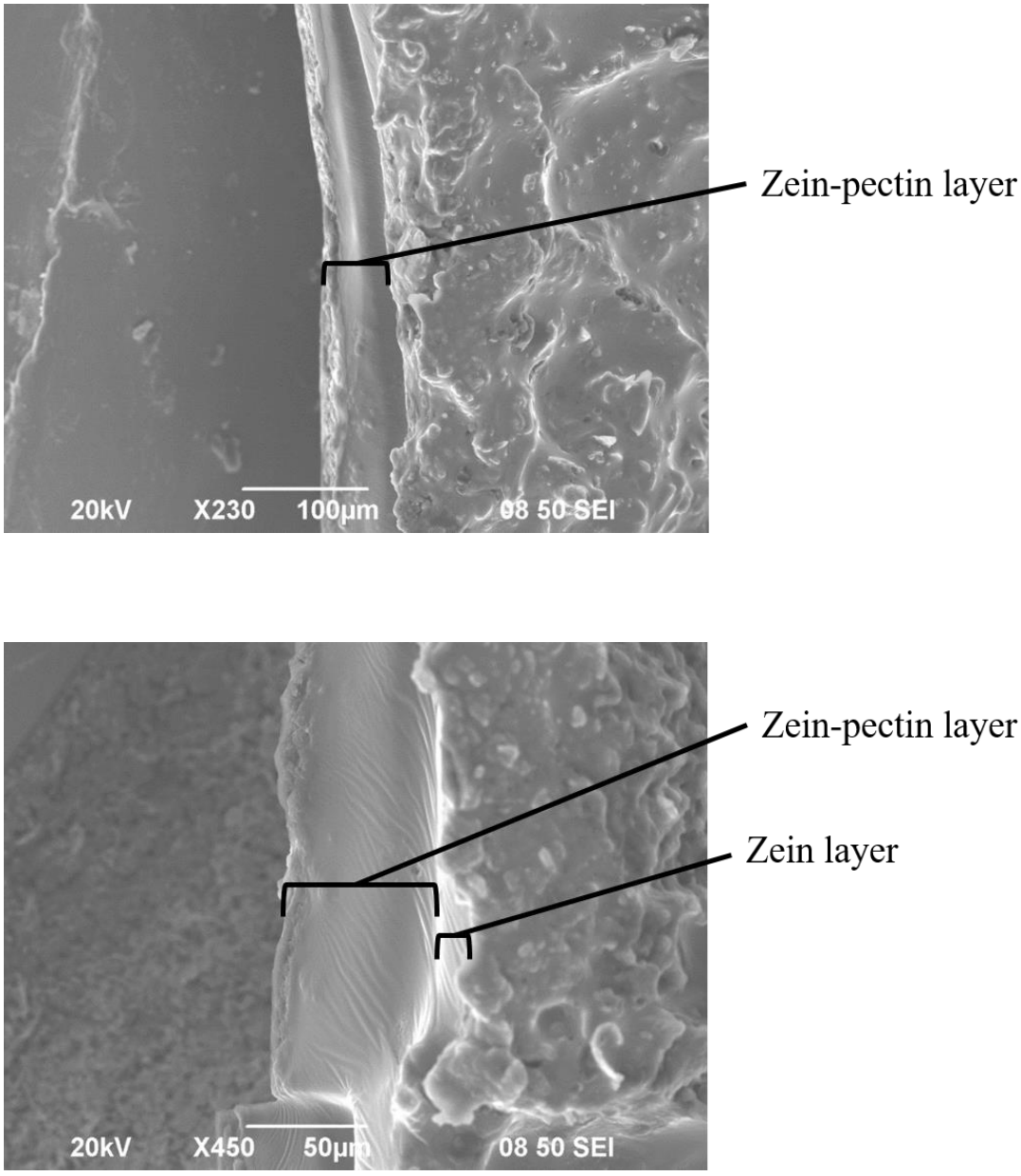

Figure 4.3 SEM images of the cross section of one layer zein-pectin and one layer zein.

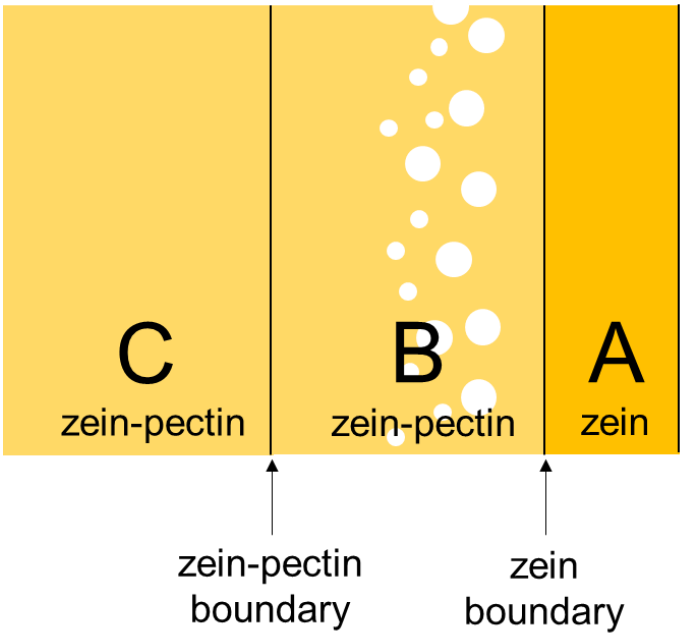

Figure 4.4 Schematic diagram of the three-layered structure of zein-pectin coated dosage form for colon-specific drug delivery. (A) The zein layer formed from 20% (w/v) zein solution. (B) The zein-pectin layer prepared by coating zein solution after pectin, with pores formed. (C) The zein-pectin layer same as layer B, without pores.

4.2.3 Swelling test

Swelling behaviors of zein, pectin, and zein-pectin films are shown in Figure 4.5, presented as swelling index versus time interval. The swelling test was performed in solution of pH 7.4 at 37 ℃ for 6 hours. Pectin swelled rapidly at the beginning and gradually attained about SI 16 after 6 hour-incubation. During the test, pectin absorbed water and became transparent because pectin was hydrophilic in nature. The hydroxyl groups of pectin formed hydrogen bonding with water molecules, resulting in water absorption of the film. Water absorption led to increase in the weight of the film. For zein and zein-pectin, they had similar swelling rate and remained steady at SI less than 1 for the whole incubation time. There was no significant change in the weight and appearance of the film, showing the zein and zein-pectin films were not water-absorbing. This suggested that zein-pectin complex formed could significantly reduce the swelling of pectin. Zein and pectin formed hydrogen bonding between their amide groups and hydroxyl groups. Suppression of the swelling of pectin by zein may be due to the occupying of the hydroxyl groups from water binding to the pectin molecules and zein having hydrophobic amino acid residues prevented the formation of hydrogen bonds between pectin and water molecules.

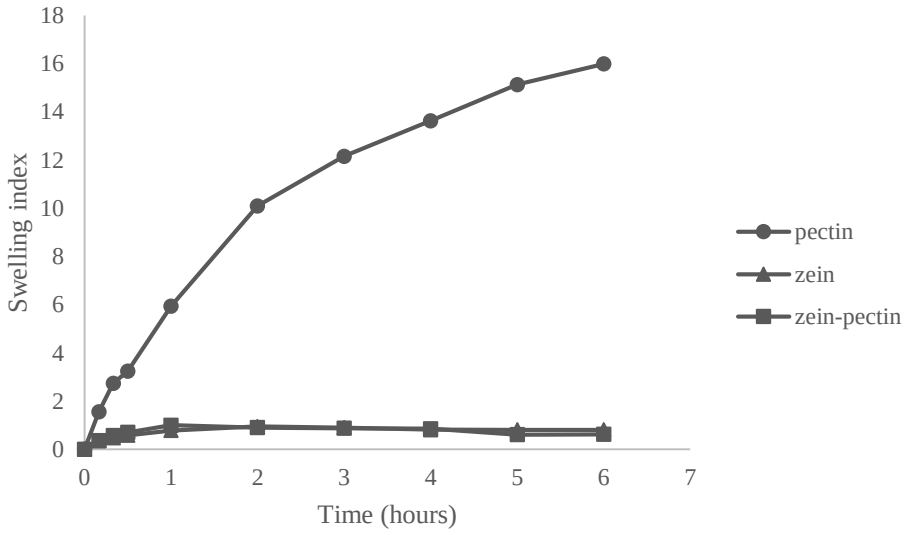

Figure 4.5 Swelling test of zein, pectin and zein-pectin films. The film samples were incubated in phosphate buffer solution of pH 7.4 at 37 ℃. For easy measurement, pectin film was cross-linked by 50 mM calcium chloride to reduce the solubility in water.

4.2.4 Dissolution studies

A zein-pectin dosage form for colon-specific delivery was prepared by dip coating technique. The release tests of the capsules were conducted in simulated gastrointestinal solution. The dosage forms were incubated in SGF for 2 hours, followed by SIF for 4 hours and SCF for 18 hours. Figure 4.6 shows the release profiles of the uncoated vitamin C tablet and the zein-pectin dosage form. In Figure 4.6, release of vitamin C to the solution was increased rapidly during the 2-hour incubation in SGF and attained 84.9% after the incubation. A plateau was observed after transferring the tablet to SIF for the release at 2-6 hours. The final release was 85.5%. This may be because the tablet was enclosed in a dialysis tubing and some vitamin C was trapped in the membrane. The tubing lacked stirring or mixing resulting in incomplete release. For the zein-pectin dosage form shown in Figure 4.7, there was no release during the 2-hour SGF release test. After transferring the capsule to the SIF, the release was approximately 0% in 4 hours. Then the capsule was transferred to SCF for the 18-hour release test. The release increased gradually from 0% to 13.5% after 2 hours in SCF. The increase continued at a constant rate until it reached 28.8% after 4 hours in SCF. The release increased slowly during 10 to 12 hours in SIF and reached 37.9% at 12 hours. After 18 hours in SCF, about 79.1% release was obtained.

The results from the dissolution studies agree with the mechanism proposed by Liu and coworkers[75]. They suggested that zein protected pectin from swelling while pectin prevented the digestion of zein by protease in the small intestinal solution. As shown in Figure 4.5, zein suppressed the swelling of pectin by forming zein-pectin complex. There was no release during the first 2-hour gastric digestion because neither zein nor pectin was digestible in stomach. For the 4-hour small intestinal digestion, almost no release was observed. In the zein-pectin layer, pectin was able to protect zein from protease digestion. Studies also showed that pectin hindered food absorption in the small intestine by preventing the contact between enzymes and food.[68] After 6 hours in stomach and small intestine, the capsule was transferred to the colonic solution, which contained pectinase. In human body, pectinase is produced by microflora in colon and is able to digest pectin. The increase in release was due to the digestion of pectin by pectinase. The drug was no longer protected by the zein-pectin coating and finally significant release was observed in the colonic solution.

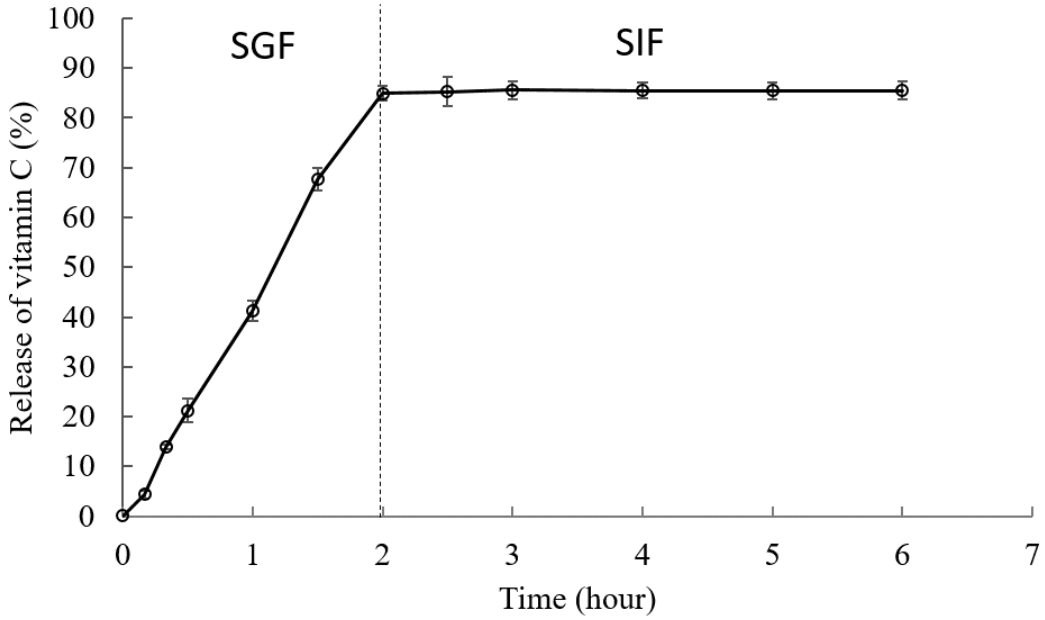

Figure 4.6 Release profile of uncoated vitamin C tablet. The release media were SGF at 0-2 hours and SIF at 2-6 hours.

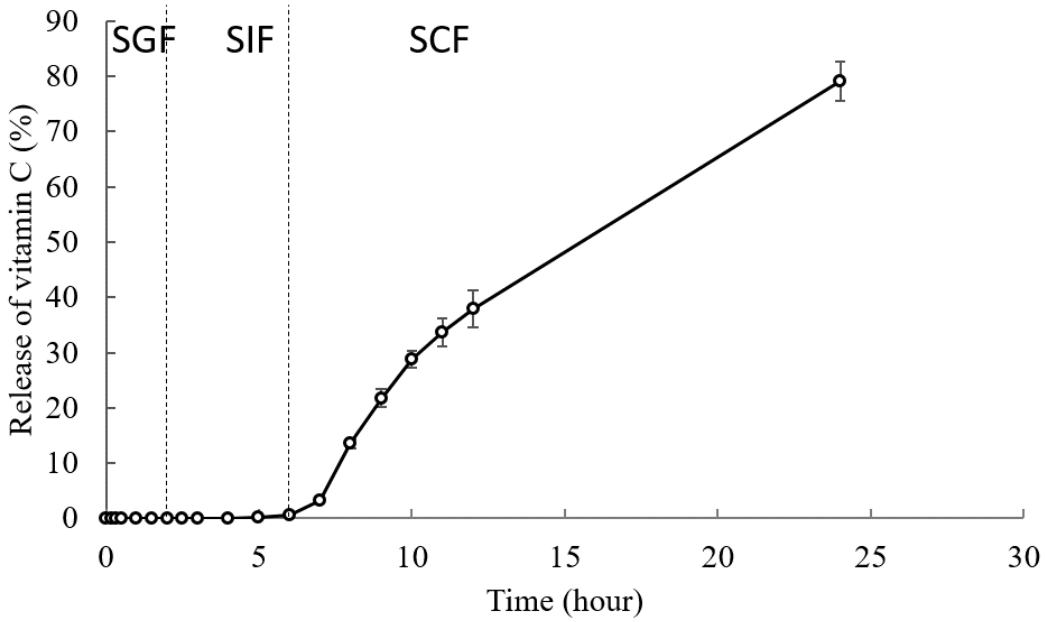

Figure 4.7 Release profile of zein-pectin coated dosage form. The release media were SGF at 0-2 hours, SIF at 2-6 hours and SCF at 6-24 hours.

4.3 Conclusions

Zein and pectin are two polymers exhibiting different physical and chemical properties. Zein is a more hydrophobic material due to the presence of the nonpolar amino acid residues. Pectin is a hydrophilic polymer which has an extensive network of hydroxyl groups. These two polymers were combined to form a complex by hydrogen bonds. The swelling of pectin was considerably suppressed by forming zein-pectin complex. This provided evidence to the explanation of the subsequent release test that zein prevented premature release of the dosage form. Zein-pectin coated dosage form for colon-specific drug delivery was successfully made with excellent controlled release property. Almost no release in the simulated upper gastrointestinal fluids indicating zein-pectin complex is an effective drug delivery system. The major factor that controls the release was the pectinase which digested pectin in the colon. For future development, zein-pectin dosage form can be modified to form a capsule for oral drug delivery encapsulating of different types of drugs.

Chapter 5 Conclusions

Oral drug delivery is the most preferable route of administration, in which one of the most popular dosage forms is capsule. Soft and hard capsules can encapsulate liquid and solid drugs and act as carriers to allow easy swallow and mask the flavors of drugs. Functional properties add values to the capsules and further extend the use of capsule in pharmaceutical industry. Controlled drug delivery is an advanced technology and provides advantages over the conventional dosage forms. Based on the gelatin capsule, controlled release can be achieved by forming enteric coating. However, the current safety issues about the gelatin have raised consumers' concern. New materials are now extensively studied and their possibility in replacing gelatin is under investigation. Among these materials, plant-based resources which are environmentally friendly and derived from natural sources are highly preferred.

In this project, the plant-derived materials were selected as the major polymers. Corn zein and pectin are biodegradable, renewable and nontoxic natural polymers. They have high customer acceptability which is one of the important competing factor in the industry. The poor flexibility of the zein films could be improved by the addition of various plasticizers. Oleic acid is favorable regarding the improvement of water barrier property, while PEG is more desirable in view of the enhancement of mechanical strength of the zein films. Glycerol is also a good candidate when it is

combined with other types of plasticizers. The disintegration ability of a capsule in different liquid media is an important criteria for selecting the suitable plasticizers. Due to the unsatisfactory results of the PEG- and glycerol-plasticized zein capsules, oleic acid-plasticized zein capsule was selected. The mechanical properties of the capsules need further improvement. Delayed release of the capsules can be achieved using zein alone as the major polymer although little release was found in SGF. Generally zein capsules having controlled release property were successfully made. Further investigation of the mechanical strength and minimizing the release in gastric solution may be required to fulfill industrial acceptability.

Colon-specific drug delivery is also an imperative topic that attracts researchers' attentions. If site-specific delivery can be developed, it allows cure of colorectal disease using smaller dosage and higher efficacy. It has been shown that delayed release in the small intestine was achieved using zein alone as the capsule material. In order to produce a colon-specific solid dosage form, a more complex system may be involved. In this project, the combined use of zein and pectin shows an excellent potential in colonic drug delivery. Zein and pectin molecules formed an interacted complex by hydrogen bonding and their combination caused certain degree of change of their secondary structures. The zein-pectin complex has improved the swelling

property of pectin by hindering the interactions between pectin and water molecules. Studies have shown that pectin prevented the contact between food and the intestinal enzymes, resulting in less food absorption. This was further proven by the results of the dissolution studies. Almost no release in the stomach and small intestine of the zein-pectin dosage form was found. By forming zein-pectin complex, pectin protected zein from protease attack and itself is resistant to gastric and intestinal digestion. As mentioned, zein prevented swelling of pectin and hence showing no premature release from the dosage form. From the results, it is found that the combination of zein and pectin is a promising system which compensates the weakness of the materials when they are used alone. The system can be further developed using different materials, such as chitosan. Chitosan is a polysaccharide derived from chitin. Similar to pectin, chitosan is also resistant to digestion in the stomach and small intestine, but can be degraded in the colon. In contrast, chitosan is a basic polysaccharide which is not soluble in water but is soluble in acidic solutions. The combination of zein and chitosan may prevent premature release in the stomach owing to the acidity of the gastric juice, and protected zein from protease digestion by forming an interacted complex between the zein and chitosan molecules.

References

1. Liu, L. S.; Fishman, M. L.; Kost, J.; Hicks, K. B., Pectin-based systems for colon-specific drug delivery via oral route. *Biomaterials* **2003,** *24* (19), 3333-3343.

2. Chen, H. M.; Langer, R., Oral particulate delivery: status and future trends. *Adv Drug Deliver Rev* **1998,** *34* (2-3), 339-350.

3. Roger, E.; Lagarce, F.; Garcion, E.; Benoit, J. P., Biopharmaceutical parameters to consider in order to alter the fate of nanocarriers after oral delivery. *Nanomedicine-Uk* **2010,** *5* (2), 287-306.

4. Ponchel, G.; Irache, J. M., Specific and non-specific bioadhesive particulate systems for oral delivery to the gastrointestinal tract. *Adv Drug Deliver Rev* **1998,** *34* (2-3), 191-219.

5. Freire, A. C.; Basit, A. W.; Choudhary, R.; Piong, C. W.; Merchant, H. A., Does sex matter? The influence of gender on gastrointestinal physiology and drug delivery. *Int J Pharmaceut* **2011,** *415* (1-2), 15-28.

6. McConnell, E. L.; Fadda, H. M.; Basit, A. W., Gut instincts: Explorations in intestinal physiology and drug delivery. *Int J Pharmaceut* **2008,** *364* (2), 213-226.

7. Garbacz, G.; Klein, S., Dissolution testing of oral modified-release dosage forms. *J Pharm Pharmacol* **2012,** *64* (7), 944-968.

8. Kalantzi, L.; Goumas, K.; Kalioras, V.; Abrahamsson, B.; Dressman, J. B.; Reppas, C., Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm Res* **2006,** *23* (1), 165-176.

9. Malagelada, J. R.; Longstreth, G. F.; Summerskill, W. H. J.; Go, V. L. W., Measurement of Gastric Functions during Digestion of Ordinary Solid Meals in Man. *Gastroenterology* **1976,** *70* (2), 203-210.

10. Dressman, J. B.; Berardi, R. R.; Dermentzoglou, L. C.; Russell, T. L.; Schmaltz, S. P.; Barnett, J. L.; Jarvenpaa, K. M., Upper Gastrointestinal (Gi) Ph in Young, Healthy-Men and Women. *Pharm Res* **1990,** *7* (7), 756-761.

11. Hofmann, A. F.; Pressman, J. H.; Code, C. F.; Witztum, K. F., Controlled Entry of Orally-Administered Drugs - Physiological Considerations. *Drug Dev Ind Pharm* **1983,** *9* (7), 1077-1109.

12. Meyer, J. H., Gastric-Emptying of Ordinary Food - Effect of Antrum on Particle-Size. *Am J Physiol* **1980,** *239* (3), G133-G135.

13. Chang, F. Y.; Lu, C. L.; Chen, C. Y.; Lee, S. D.; Doong, M. L.; Wang, P. S., Distal stomach appears essential in the regulation of gastrointestinal transit. *J Gastroenterol* **2000,** *35* (6), 424-428.

14. Wilding, I. R.; Coupe, A. J.; Davis, S. S., The role of gamma-scintigraphy in oral

drug delivery. *Adv Drug Deliver Rev* **2001,** *46* (1-3), 103-124.

15. Evans, D. F.; Pye, G.; Bramley, R.; Clark, A. G.; Dyson, T. J.; Hardcastle, J. D., Measurement of Gastrointestinal Ph Profiles in Normal Ambulant Human-Subjects. *Gut* **1988,** *29* (8), 1035-1041.

16. Fallingborg, J.; Christensen, L. A.; Ingemannielsen, M.; Jacobsen, B. A.; Abildgaard, K.; Rasmussen, H. H., Ph-Profile and Regional Transit Times of the Normal Gut Measured by a Radiotelemetry Device. *Aliment Pharm Therap* **1989,** *3* (6), 605-613.

17. Schiller, C.; Frohlich, C. P.; Giessmann, T.; Siegmund, W.; Monnikes, H.; Hosten, N.; Weitschies, W., Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Aliment Pharm Therap* **2005,** *22* (10), 971-979.

18. Davis, S. S.; Hardy, J. G.; Fara, J. W., Transit of Pharmaceutical Dosage Forms through the Small-Intestine. *Gut* **1986,** *27* (8), 886-892.

19. Kerlin, P.; Phillips, S., Variability of Motility of the Ileum and Jejunum in Healthy Humans. *Gastroenterology* **1982,** *82* (4), 694-700.

20. Indireshkumar, K.; Brasseur, J. G.; Faas, H.; Hebbard, G. S.; Kunz, P.; Dent, J.; Feinle, C.; Li, M. J.; Boesiger, P.; Fried, M.; Schwizer, W., Relative contributions of "pressure pump" and "peristaltic pump" to gastric emptying. *Am J Physiol-Gastr L* **2000,** *278* (4), G604-G616.

21. Varum, F. J. O.; Merchant, H. A.; Basit, A. W., Oral modified-release formulations in motion: The relationship between gastrointestinal transit and drug absorption. *Int J Pharmaceut* **2010,** *395* (1-2), 26-36.

22. Caspary, W. F., Physiology and Pathophysiology of Intestinal-Absorption. *Am J Clin Nutr* **1992,** *55* (1), 299-308.

23. Fallingborg, J., Intraluminal pH of the human gastrointestinal tract. *Dan Med Bull* **1999,** *46* (3), 183-196.

24. Press, A. G.; Hauptmann, I. A.; Hauptmann, L.; Fuchs, B.; Fuchs, M.; Ewe, K.; Ramadori, G., Gastrointestinal pH profiles in patients with inflammatory bowel disease. *Aliment Pharm Therap* **1998,** *12* (7), 673-678.

25. Diakidou, A.; Vertzoni, M.; Goumas, K.; Soderlind, E.; Abrahamsson, B.; Dressman, J.; Reppas, C., Characterization of the Contents of Ascending Colon to Which Drugs are Exposed After Oral Administration to Healthy Adults. *Pharm Res* **2009,** *26* (9), 2141-2151.

26. Coupe, A. J.; Davis, S. S.; Wilding, I. R., Variation in Gastrointestinal Transit of Pharmaceutical Dosage Forms in Healthy-Subjects. *Pharm Res* **1991,** *8* (3), 360-364.

27. Mcneil, N. I.; Bingham, S.; Cole, T. J.; Grant, A. M.; Cummings, J. H., Diet and Health of People with an Ileostomy .2. Ileostomy Function and Nutritional State. *Brit J Nutr* **1982,** *47* (3), 407-415.

28. Akerlund, J. E.; Bjorkhem, I.; Angelin, B.; Liljeqvist, L.; Einarsson, K., Apparent Selective Bile-Acid Malabsorption as a Consequence of Ileal Exclusion - Effects on Bile-Acid, Cholesterol, and Lipoprotein Metabolism. *Gut* **1994,** *35* (8), 1116-1120.

29. Vassallo, M.; Camilleri, M.; Phillips, S. F.; Brown, M. L.; Chapman, N. J.; Thomforde, G. M., Transit through the Proximal Colon Influences Stool Weight in the Irritable-Bowel-Syndrome. *Gastroenterology* **1992,** *102* (1), 102-108.

30. Fallingborg, J.; Christensen, L. A.; Jacobsen, B. A.; Rasmussen, S. N., Very-Low Intraluminal Colonic Ph in Patients with Active Ulcerative-Colitis. *Digest Dis Sci* **1993,** *38* (11), 1989-1993.

31. Catteau, D.; Barthelemy, C.; Deveaux, M.; Robert, H.; Trublin, F.; Marchandise, X.; Vandrunen, H., Contribution of Scintigraphy to Verify the Reliability of Different Preparation Processes for Enteric-Coated Capsules. *Eur J Drug Metab Ph* **1994,** *19* (2), 91-98.

32. Barker, R.; Abrahamsson, B.; Kruusmagi, M., Application and Validation of an Advanced Gastrointestinal In Vitro Model for the Evaluation of Drug Product Performance in Pharmaceutical Development. *J Pharm Sci-Us* **2014,** *103* (11), 3704-3712.

33. Choe, S. Y.; Neudeck, B. L.; Welage, L. S.; Amidon, G. E.; Barnett, J. L.; Amidon, G. L., Novel method to assess gastric emptying in humans: the Pellet Gastric Emptying

Test. *European Journal of Pharmaceutical Sciences* **2001,** *14* (4), 347-353.

34. Liu, L. S.; Fishman, M. L.; Hicks, K. B., Pectin in controlled drug delivery - a review. *Cellulose* **2007,** *14* (1), 15-24.

35. Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M., Polymeric systems for controlled drug release. *Chem Rev* **1999,** *99* (11), 3181-3198.

36. Ambade, A. V.; Savariar, E. N.; Thayumanavan, S., Dendrimeric micelles for controlled drug release and targeted delivery. *Mol Pharmaceut* **2005,** *2* (4), 264-272.

37. Pannier, A. K.; Shea, L. D., Controlled release systems for DNA delivery. *Mol Ther* **2004,** *10* (1), 19-26.

38. Roy, K.; Mao, H. Q.; Huang, S. K.; Leong, K. W., Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nat Med* **1999,** *5* (4), 387-391.

39. Anal, A. K.; Bhopatkar, D.; Tokura, S.; Tamura, H.; Stevens, W. F., Chitosan-alginate multilayer beads for gastric passage and controlled intestinal release of protein. *Drug Dev Ind Pharm* **2003,** *29* (6), 713-724.

40. Chen, S. C.; Wu, Y. C.; Mi, F. L.; Lin, Y. H.; Yu, L. C.; Sung, H. W., A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery. *J Control Release* **2004,** *96* (2), 285-300.

41. Friend, D. R., New oral delivery systems for treatment of inflammatory bowel

disease. *Adv Drug Deliver Rev* **2005,** *57* (2), 247-265.

42. Yang, L. B.; Chu, J. S.; Fix, J. A., Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. *Int J Pharmaceut* **2002,** *235* (1-2), 1-15.

43. Leopold, C. S., Coated dosage forms for colon-specific drug delivery. *Pharm Sci Technol To* **1999,** *2* (5), 197-204.

44. Steed, K. P.; Hooper, G.; Monti, N.; Benedetti, M. S.; Fornasini, G.; Wilding, I. R., The use of pharmacoscintigraphy to focus the development strategy for a novel 5-ASA colon targeting system ("TIME CLOCK (R)" system). *J Control Release* **1997,** *49* (2-3), 115-122.

45. Chourasia, M. K.; Jain, S. K., Polysaccharides for colon targeted drug delivery. *Drug Deliv* **2004,** *11* (2), 129-148.

46. Gupta, V. K.; Assmus, M. W.; Beckert, T. E.; Price, J. C., A novel pH- and time-based multi-unit potential colonic drug delivery system. II. Optimization of multiple response variables. *Int J Pharmaceut* **2001,** *213* (1-2), 93-102.

47. Gupta, V. K.; Beckert, T. E.; Price, J. C., A novel pH- and time-based multi-unit potential colonic drug delivery system. I. Development. *Int J Pharmaceut* **2001,** *213* (1-2), 83-91.

48. Fukui, E.; Miyamura, N.; Kobayashi, M., An in vitro investigation of the suitability of press-coated tablets with hydroxypropylmethylcellulose acetate

succinate (HPMCAS) and hydrophobic additives in the outer shell for colon targeting. *J Control Release* **2001,** *70* (1-2), 97-107.

49. Chourasia, M. K.; Jain, S. K., Pharmaceutical approaches to colon targeted drug delivery systems. *J Pharm Pharm Sci* **2003,** *6* (1), 33-66.

50. McConnell, E. L.; Liu, F.; Basit, A. W., Colonic treatments and targets: issues and opportunities. *J Drug Target* **2009,** *17* (5), 335-363.

51. Kosaraju, S. L., Colon targeted delivery systems: Review of polysaccharides for encapsulation and delivery. *Crit Rev Food Sci* **2005,** *45* (4), 251-258.

52. Muraoka, M.; Hu, Z. P.; Shimokawa, T.; Sekino, S.; Kurogoshi, R.; Kuboi, Y.; Yoshikawa, Y.; Takada, K., Evaluation of intestinal pressure-controlled colon delivery capsule containing caffeine as a model drug in human volunteers. *J Control Release* **1998,** *52* (1-2), 119-129.

53. Shukla, R.; Cheryan, M., Zein: the industrial protein from corn. *Ind Crop Prod* **2001,** *13* (3), 171-192.

54. Luo, Y. C.; Wang, Q., Zein-Based Micro- and Nano-Particles for Drug and Nutrient Delivery: A Review. *J Appl Polym Sci* **2014,** *131* (16).

55. Matsushima, N.; Danno, G.; Takezawa, H.; Izumi, Y., Three-dimensional structure of maize alpha-zein proteins studied by small-angle X-ray scattering. *Bba-Protein Struct M* **1997,** *1339* (1), 14-22.

56. Guo, Y. C.; Liu, Z. D.; An, H. J.; Li, M. Q.; Hu, J., Nano-structure and properties of maize zein studied by atomic force microscopy. *J Cereal Sci* **2005,** *41* (3), 277-281.

57. Lawton, J. W., Zein: A history of processing and use. *Cereal Chem* **2002,** *79* (1), 1-18.

58. Argos, P.; Pedersen, K.; Marks, M. D.; Larkins, B. A., A Structural Model for Maize Zein Proteins. *J Biol Chem* **1982,** *257* (17), 9984-9990.

59. Wang, Y.; Padua, G. W., Nanoscale Characterization of Zein Self-Assembly. *Langmuir* **2012,** *28* (5), 2429-2435.

60. Wang, Y.; Padua, G. W., Formation of Zein Microphases in Ethanol-Water. *Langmuir* **2010,** *26* (15), 12897-12901.

61. Anderson, T. J.; Lamsal, B. P., Zein Extraction from Corn, Corn Products, and Coproducts and Modifications for Various Applications: A Review. *Cereal Chem* **2011,** *88* (2), 159-173.

62. Sessa, D. J.; Mohamed, A.; Byars, J. A.; Hamaker, S. A. H.; Selling, G. W., Properties of films from corn zein reacted with glutaraldehyde. *J Appl Polym Sci* **2007,** *105* (5), 2877-2883.

63. Parris, N.; Coffin, D. R., Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *J Agr Food Chem* **1997,** *45* (5), 1596-1599.

64. Yang, Y. Q.; Wang, L. M.; Li, S. Q., Formaldehyde-free zein fiber - Preparation and investigation. *J Appl Polym Sci* **1996,** *59* (3), 433-441.

65. Liu, X. M.; Sun, Q. S.; Wang, H. J.; Zhang, L.; Wang, J. Y., Microspheres of corn protein, zein, for an ivermectin drug delivery system. *Biomaterials* **2005,** *26* (1), 109-115.

66. Lee, S. H.; Hamaker, B. R., Cys155 of 27 kDa maize gamma-zein is a key amino acid to improve its in vitro digestibility. *Febs Lett* **2006,** *580* (25), 5803-5806.

67. Mohnen, D., Pectin structure and biosynthesis. *Curr Opin Plant Biol* **2008,** *11* (3), 266-277.

68. Thakur, B. R.; Singh, R. K.; Handa, A. K., Chemistry and uses of pectin - A review. *Crit Rev Food Sci* **1997,** *37* (1), 47-73.

69. Willats, W. G. T.; McCartney, L.; Mackie, W.; Knox, J. P., Pectin: cell biology and prospects for functional analysis. *Plant Mol Biol* **2001,** *47* (1-2), 9-27.

70. Meneguin, A. B.; Cury, B. S. F.; Evangelista, R. C., Films from resistant starch-pectin dispersions intended for colonic drug delivery. *Carbohyd Polym* **2014,** *99*, 140-149.

71. Jung, J. Y.; Arnold, R. D.; Wicker, L., Pectin and charge modified pectin hydrogel beads as a colon-targeted drug delivery carrier. *Colloid Surface B* **2013,** *104*, 116-121.

72. Das, S.; Ng, K. Y., Colon-specific delivery of resveratrol: Optimization of multi-

particulate calcium-pectinate carrier. *Int J Pharmaceut* **2010,** *385* (1-2), 20-28.

73. Feng, T. J.; Keller, L. R.; Wu, P.; Xu, Y. F., An Empirical Study of the Toxic Capsule Crisis in China: Risk Perceptions and Behavioral Responses. *Risk Anal* **2014,** *34* (4), 698-710.

74. Cole, E. T.; Scott, R. A.; Connor, A. L.; Wilding, I. R.; Petereit, H. U.; Schminke, C.; Beckert, T.; Cade, D., Enteric coated HPMC capsules designed to achieve intestinal targeting. *Int J Pharmaceut* **2002,** *231* (1), 83-95.

75. Liu, L.; Fishman, M. L.; Hicks, K. B.; Kende, M.; Ruthel, G., Pectin/zein beads for potential colon-specific drug delivery: synthesis and in vitro evaluation. *Drug Deliv* **2006,** *13* (6), 417-23.

76. Desai, K. G. H.; Park, H. J., Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying. *J Microencapsul* **2005,** *22* (2), 179-192.

77. Jacobs, E. J.; Connell, C. J.; Patel, A. V.; Chao, A.; Rodriguez, C.; Seymour, J.; McCullough, M. L.; Calle, E. E.; Thun, M. J., Vitamin C and vitamin E supplement use and colorectal cancer mortality in a large American Cancer Society cohort. *Cancer Epidem Biomar* **2001,** *10* (1), 17-23.

78. Kshirsagar, S. J.; Bhalekar, M. R.; Pawar, R. P., In vitro drug release and in vivo human X-ray studies of ileo-cecal targeting budesonide fast disintegrating tablet. *Drug*

Dev Ind Pharm **2009,** *35* (7), 788-795.

79. Hu, L. D.; Li, L.; Luo, Z. L.; Yang, J. X.; Liu, W., Determination of Trace Vitamin C by Ion-Pair HPLC with UV Detection in Calcium Gluconate and Vitamin C Compound Oral Solution. *J Chromatogr Sci* **2012,** *50* (2), 102-107.

80. Santosa, F. X. B.; Padua, G. W., Tensile properties and water absorption of zein sheets plasticized with oleic and linoleic acids. *J Agr Food Chem* **1999,** *47* (5), 2070-2074.

81. Cao, N.; Yang, X. M.; Fu, Y. H., Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films. *Food Hydrocolloid* **2009,** *23* (3), 729-735.

82. Fabra, M. J.; Talens, P.; Chiralt, A., Tensile properties and water vapor permeability of sodium caseinate films containing oleic acid-beeswax mixtures. *J Food Eng* **2008,** *85* (3), 393-400.

83. Yahya, M. Z. A.; Arof, A. K., Effect of oleic acid plasticizer on chitosan-lithium acetate solid polymer electrolytes. *Eur Polym J* **2003,** *39* (5), 897-902.

84. Lai, H. M.; Padua, G. W., Properties and microstructure of plasticized zein films. *Cereal Chem* **1997,** *74* (6), 771-775.

85. Suyatma, N. E.; Tighzert, L.; Copinet, A., Effects of hydrophilic plasticizers on mechanical, thermal, and surface properties of chitosan films. *J Agr Food Chem* **2005,**

53 (10), 3950-3957.

86. Ge, H. H.; Yang, F.; Hao, Y. P.; Wu, G. F.; Zhang, H. L.; Dong, L. S., Thermal, Mechanical, and Rheological Properties of Plasticized Poly(L-lactic acid). *J Appl Polym Sci* **2013,** *127* (4), 2832-2839.

87. Baiardo, M.; Frisoni, G.; Scandola, M.; Rimelen, M.; Lips, D.; Ruffieux, K.; Wintermantel, E., Thermal and mechanical properties of plasticized poly(L-lactic acid). *J Appl Polym Sci* **2003,** *90* (7), 1731-1738.

88. Tillekeratne, M.; Easteal, A. J., Modification of zein films by incorporation of poly(ethylene glycol)s. *Polym Int* **2000,** *49* (1), 127-134.

89. Gao, C. L.; Stading, M.; Wellner, N.; Parker, M. L.; Noel, T. R.; Mills, E. N. C.; Belton, P. S., Plasticization of a protein-based film by glycerol: A spectroscopic, mechanical, and thermal study. *J Agr Food Chem* **2006,** *54* (13), 4611-4616.

90. Xu, H.; Chai, Y.; Zhang, G., Synergistic effect of oleic acid and glycerol on zein film plasticization. *J Agric Food Chem* **2012,** *60* (40), 10075-81.

91. Barth, A., Infrared spectroscopy of proteins. *Biochim Biophys Acta* **2007,** *1767* (9), 1073-101.

92. Mizuno, A.; Mitsuiki, M.; Motoki, M.; Ebisawa, K.; Suzuki, E., Relationship between the glass transition of soy protein and molecular structure. *J Agric Food Chem* **2000,** *48* (8), 3292-7.